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Editorial
The threats of antimicrobial drug resistance and the need for stewardship initiative
Baye Gelaw

Following the widespread use of antimicrobial drugs, human pathogens resistant to these agents were isolated. Even when new agents are introduced into clinical practice, resistance to these drugs will certainly appear. For example, despite encountering virtually no case of resistance in clinical trials with the novel antimicrobial daptomycin, case reports of resistance to this agent appeared shortly after its introduction into clinical use (1). The control of infectious diseases is seriously threatened by the steady increase in the number of microorganisms that are resistant to antimicrobial drugs. Infection by such microbes leads to increased morbidity and mortality (2).

The risk of transmission of antibiotic resistant (AR) bacteria from one country to another becomes a global concern. Drug resistant microbial strains can be imported into a country and disseminated before their presence is even recognized (3). The problem of antimicrobial resistance has no boundaries. However, for many bacterial genera, the problem of antibiotic resistance is more pronounced in developing countries (4). In Nigeria, studies reported as many as 88% Staphylococcus aureus infections cannot be treated with methicillin. Methicillin resistant Staphylococcus aureus infection was also reported as an acute problem in the emerging economies known as the “BRICS” states: Brazil, Russia, India, China and South Africa (5). In 2012, the WHO reported a gradual increase in resistance to HIV drugs. About 480,000 new cases of multi-drug resistance (MDR-TB) tuberculosis was also reported by WHO in 2013 and extensively drug-resistant tuberculosis (XDR-TB) has been identified in 100 countries.

Drug resistant bacteria pathogens have been reported in different hospitals in Ethiopia. Recent reports from Gondar showed that a significant increase of ciprofloxacin resistance ranged from 0% in 2002 to 30%, 24.4%, and 30% in 2012 for E. coli, Klebsiella species and Citrobacter species, respectively (6). The prevalence of methicillin resistant Staphylococcus aureus isolates from surgical sites was reported 34.6% and that of Streptococcus pneumoniae taken from the nasopharyngeal swabs of children was reported 33.2% (7). In Arba Minch hospital, Southern Ethiopia, most of the Staphylococcus aureus and Gram negative bacterial isolates taken from adult patients with pneumonia were resistant to tetracycline (100%), Penicillin (83.3%), Ampicillin (50-100%), Doxycycline (50-100%), and Trimethoprim-sulfamethoxazole (83.3%). Multi-drug resistance was reported 60.3% (8). Moreover, tuberculosis primary drug resistance was found 15.6% in Addis Ababa (9). The national TB drug resistance survey conducted in 2005 documented a 1.6% and 11.8% prevalence of MDR-TB among new and previously treated TB patients, respectively (10). Moreover, in the 2010 national TB drug resistance surveillance report, 2.3% of new TB cases and 17.8% of previously treated TB cases were estimated to have MDR (11).

Drug resistances are triggered by a number of factors such as miss use of antibiotics, inappropriate prescriptions and/or dosing, and poor patient adherence (12). Improved surveillance of resistant pathogens and development of new antimicrobial drugs and rational use are the main strategies against antimicrobial resistance (13). In addition, a growing body of evidence demonstrates that hospital based programs dedicated to improving antibiotic use, commonly referred to as “antimicrobial stewardship” programs (AMSPs) can optimize the treatment of infections and reduce adverse events (14).

AMSPs refers to a set of coordinated strategies to improve the use of antimicrobial medications with the goal of enhancing patient health outcomes, reducing drug resistance, lessen the risk of adverse effects, and decreasing unnecessary cost (15). The core elements of hospital AMSP include leadership commitment, accountability, drug expertise, action, tracking, reporting and education. Essential to a successful AMSP is the presence of at least one infectious disease-trained Physician who dedicates to design, implementation, and
function of the program. The clinical microbiology laboratory is a key component in
the function of AMSP that summarize data on AR rates which allow the AMSP team to
determine the burden of AR in the hospital. The team also composed of a pharmacist that will
determine the cost-effectiveness of antimicrobial drugs. Infection control staff gathers data on
nosocomial infections which may assist in the evaluation of the outcomes of the strategies
of the AMSP’s strategies. Moreover, passive endorsement of hospital administrators may
facilitate for program funding, utilize institutions policy and award autonomy for the AMSP
committee (16).

In conclusion, available data suggest that AR has reached unacceptable level in developing
countries. AMSP need an extensive effort at the individual and institutional levels. Commitment
to implementation of AMSP must come from the highest levels of hospital administration,
along with a willingness to invest resources in program development. Moreover, national and
international policy decisions backed by political and social willpower are necessary to provide a
more accurate assessment and prevention of antimicrobial resistance.

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Rotavirus and Associated Risk Factors among Under Five Children with Acute Diarrhea in Addis Ababa, Ethiopia
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Abstract
Background: Rotavirus is the most common cause of severe gastroenteritis (GE) among under five children. Ethiopia is one of the five countries with the greatest Rotavirus burden worldwide. So, determining prevalence of Rotavirus among under five children with diarrhea is crucial.
Methods: In this cross-sectional study, 246 stool samples were collected consecutively from under five children with diarrhea in the previous 10 days from December 2013- March 2014. Samples were investigated for Rotavirus antigen by Enzyme Immuno-Assay technique. Child and guardian related information was collected using structured and pre-validated questionnaire. Data was entered into Epi-Info version 3.5.4 and analyzed using SPSS version 20 software.
Result: Of 246 children with diarrhea, 85 (34.6%) children were positive for Rotavirus antigen and no Rotavirus antigen was detected in the rest 161 children. The Rotavirus infection was not significantly associated with any potential risk factors in this study. Conclusion: -The higher prevalence of Rotavirus found in this study shows that the infection is an important public health problem in Addis Ababa, Ethiopia. Therefore, early age vaccination should be strengthened to prevent mortality and morbidity due to Rotavirus infection. Further study is essential to see the burden of disease in other regions of Ethiopia.
Keywords: Diarrhea, Children, Rotavirus, EIA, Addis Ababa (Ethiopia).

Background
Diarrhea is the second leading cause of death among children under five years of age globally. Infectious diarrhea can be caused by a wide range of viruses, bacteria and parasites. In both developed and developing countries, viruses are the leading cause of acute diarrhea (1).

Acute gastroenteritis (AGE) caused by Rotavirus is a serious global problem associated with considerable morbidity and mortality among infants and young children. Rotavirus is responsible for about 2 million severe illnesses and up to 50% of hospitalizations due to severe diarrhea among under five children worldwide. It is estimated to cause 527,000 deaths in children annually and 85% of these deaths occur in low income countries in South Asia and sub-Saharan Africa (2–7).

Reports showed that about 6% of all Rotavirus related deaths globally occur in Ethiopia. Rotavirus kills more than 28,000 under five children each year in the country. It is responsible for an estimated 28% of hospitalizations due to diarrhea (7–9).
Rotavirus infection is not routinely diagnosed in most Ethiopian hospitals. This is due to the unaffordable cost of diagnosis. There are limited data on the burden and potential risk factors of Rotavirus. This study aimed to determine the prevalence of Rotavirus and possible risk factors among children with diarrhea in Addis Ababa, Ethiopia.

Methods
Study Setting
A cross-sectional study was conducted in four health centers (Selam, Kolfe, Semen and Addis Ketema) of Addis Ababa from December 2013 to March 2014. Based on 2007 census, the population of Addis Ababa was estimated 2.7 million with 195,932 under five children (11).

Specimen collection
A total of 246 stool samples were collected from under five children with the symptom of AGE. Onset of AGE within ten days, age of children less than five years, agreement of parents/caregivers to participate and children who provide adequate stool were the inclusion criteria.

Consented caregivers were interviewed using structured, pre-tested questionnaire and requested to bring about 3ml of stool specimen. Specimens were collected using clean, labeled and screw capped container, and transported with cold-chain system to the Virology Research Laboratory at Ethiopian Public Health Institute (EPHI) within an hour of collection.

Sample Processing
All 246 stool samples were tested for Rotavirus antigen using ProSpecT Rotavirus Enzyme Immuno-Assay (EIA) kit (Oxoid Ltd Company, UK). This kit is specific to group Rotaviruses and had 100% sensitivity and 99.2% specificity for Rotavirus antigens in stool. A 10% suspension of stool specimen was prepared by mixing 100μl (two drops) of liquid stool and 1ml of sample diluent. The mixture was homogenized by vortex mixer and allowed to settle for 15 minutes. 100μl stool suspension was taken from the supernatant and added to antibody pre-coated EIA plates. Once all the test and control samples were added, 100μl of conjugate was added and incubated for an hour at room temperature. The EIA plate was washed five times by EIA washer with diluted wash buffer (1:5). The plate was inverted and tapped on absorbent paper to avoid last traces of wash buffer remained. Then 100μl substrate was added to each well and incubated for 10 minutes. Finally enzyme substrate reaction was stopped by adding 100μl stop solution (0.46 mol/L sulpheric acid). The color intensity of wells was read by ELISA plate reader at 450 nm within 30 minutes from addition of stop solution. The cutoff value was determined by adding 0.2 to the negative control optical density (OD). The result of a test sample was recorded as positive when the OD was greater than the cutoff value and negative if lower.

Statistical Analysis
The data were coded, cleaned and entered using Epi Info software version 3.5.4. Analysis was done by IBM SPSS version 20 (IBM Corp. Armonk, NY). Binary logistic regression analysis was used to assess the possible associated factors. Results were interpreted based on 95% confidence level and odds ratio was used to see the strength of association between dependent and independent variables. P-value less than 0.05 were considered as statistically significant.

Ethical Considerations
The study was approved by Ethics Review Committee of the department of Medical Laboratory Sciences, Addis Ababa University and EPHI. Informed consent was obtained from a caregiver or guardian. Positive results were reported to the respective clinicians as soon as possible.

Results
Socio-demographic and clinical Characteristics
In this study, the proportion of male children was 51.6% (n=127) and that of females 48.4% (n=119) making a male to female ratio of 1.07. The mean age of the children was 28.5 months with standard deviation (SD) of ± 15.5. One hundred twenty-five children (51%) were within the age group 25-59 months. Seventy-four children attended day care service but only 10 were at exclusive breast feeding. All enrolled children had diarrhea for 1-10 days with a mean of diarrheal disease for 3 days. The proportion of children whom had sign and symptoms was 66.3%, 54.1%, 47.2%, and 19.1% abdominal pain, vomiting, fever, and tenesmus respectively. The mean episodes of vomiting were 2.2 per day (ranges 1 to 10 episodes).
Prevalence of Rotavirus infection
The overall prevalence of Rotavirus infection was 34.6% (N=85/246). The majority of confirmed Rotavirus cases were among females and 48.2% Rotavirus infection occurred at the age of 25-59 months, followed by 35.3% in 13-24 month of age children. Children less than 6 month of age and those with the age group between 7-12 months had the lowest prevalence of Rotavirus infection, 8.2% each (Fig 1). However, neither sex nor age group had any significant association with Rotavirus infection (p>0.05) (Table 1).

![Figure 1: Frequency of Rotavirus infection by age of children in months, Addis Ababa, Ethiopia, 2014](image)

Among all Rotavirus positive cases, 61.2% had vomiting, and 66%, 20%, and 43.5% had abdominal pain, tenesmus and fever respectively. The majority (98%) of Rotavirus infected children had onset of diarrhea within 7 days and 90% of them had 1-5 diarrhea episodes per day. In this study, there was no significant association between the guardian educational level, employment type, income and family related factors.
Table 1: Rotavirus detection and its association with possible risk factors of children and their guardian in Addis Ababa, Ethiopia, 2013-2014 (N=246)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Rota EIA Result</th>
<th>COR (95% C.I.)</th>
<th>P-value</th>
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<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
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<td>41</td>
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<tr>
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<td>Female</td>
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<td>Age in months</td>
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<td>13</td>
<td>1</td>
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<td></td>
<td>7-12</td>
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<td>15</td>
<td>0.906 (0.336-2.44)</td>
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<td>13-24</td>
<td>30</td>
<td>49</td>
<td>1.05 (0.396-2.76)</td>
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<td></td>
<td>25-59</td>
<td>41</td>
<td>84</td>
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<td>No</td>
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<td>115</td>
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</tr>
<tr>
<td>Feeding Practice</td>
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<td>53</td>
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<td>Solid Food Only</td>
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<td>Cow Milk</td>
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<tr>
<td>Sex of Caregiver</td>
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<td>15</td>
<td>0.87 (0.36-2.1)</td>
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<tr>
<td></td>
<td>Female</td>
<td>76</td>
<td>146</td>
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<tr>
<td>Caregiver Age</td>
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<td>29</td>
<td>1 (0.4-2.6)</td>
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<td>25-35</td>
<td>56</td>
<td>112</td>
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<td>36-57</td>
<td>12</td>
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<td>Caregiver Education</td>
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<td>15</td>
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<td>75</td>
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<tr>
<td>Level</td>
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<td>Higher</td>
<td>9</td>
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<tr>
<td>Extra Children</td>
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<td>Children</td>
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<td>45</td>
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<td>Occupation</td>
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<td></td>
<td>Private</td>
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<td>61</td>
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<td></td>
<td>Merchant</td>
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<td>14</td>
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<td></td>
<td>House wife</td>
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<td>0.94 (0.3-2.7)</td>
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<td>85</td>
<td>0.813 (0.465-1.4)</td>
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<tr>
<td></td>
<td>&gt;1228</td>
<td>30</td>
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<tr>
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<td>Piped</td>
<td>83</td>
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<td>1</td>
<td>3.8 (0.345-43)</td>
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<td></td>
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<td>83</td>
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<td>3</td>
<td>1.3 (0.21-7.7)</td>
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<td>1</td>
<td>3.8 (0.345-43)</td>
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<td>160</td>
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<tr>
<td>Collectors</td>
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<td>156</td>
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Discussion
Annually, an estimated 2.5 billion cases and 1.5 million deaths of diarrhea occur among under five children, contributing one in every five deaths. More than half of these cases and 80% of deaths occur in Africa and South Asia. Ethiopia is the fifth highest country that contributes for under-five death due to diarrhea (12). In this country, diarrhea is responsible for 230,000 deaths annually. Rotavirus is responsible for an estimated 28% of all under-five diarrhea hospitalizations in Ethiopia (7, 9).

The present study revealed that Rotavirus accounted for a 34.6% of all diarrhea cases. This implies that large proportions of children are suffering from this disease in the study area. Previous WHO and UNICEF reports showed that Rotavirus is responsible for up to 40% of hospital admissions due to diarrhea among under five children worldwide (12).

The prevalence of Rotavirus infection in the current study was higher than the findings of other previous studies conducted in Ethiopia. For example, Almaze, Tson et al., and Yassin reported Rotavirus prevalence of 20% in Addis Ababa, 26.6% in Jimma and 22% in Hawassa (13-15). This might be due to variation in geographic location and seasonal variation during sample collection and investigation. On the other hand, the current Rotavirus infection prevalence is nearly similar to the prevalence report in Sudan (36%) (16), Italy (34.9%) (17), China (33.7%) (18) and Nepal (36.6%) (19). This study reports may indicate Rotavirus infection is a challenge both for developed and developing countries. Nevertheless, the result of the current study was lower than reports from some African and Asian countries. For instance, the prevalence of Rotavirus infection was reported 45.5% in Uganda (20), 58% in Ghana (21), 44.6% in Egypt (22), and 42.8% in Cameroon (23). On the other hand, higher prevalence was reported in Saudi Arabia (65.5%) (24) and Pakistan (67%) (2).

In the current study, Rotavirus was detected throughout all age groups (2-59 months). This result was in agreement with the findings at Jimma, Ethiopia [14] and India [25] but different from previous findings in Addis Ababa Ethiopia [13] and Ivory Coast [26] where children 6-12 months were more affected. This might be related to the child feeding practice habit and hygienic behavior of mothers. The epidemiological features of rotavirus infection may be quite relevant for evaluation of the performance of a rotavirus vaccine in different settings, as well as for monitoring its impact during vaccination under routine conditions. Rotavirus disease occurs year-round, with a slight seasonal pattern. Eighty-five percent of rotavirus-positive diarrhea episodes, as well as 86% of cases of dehydration due to rotavirus, occurred during the first year of life. However, rotavirus illnesses occur less commonly during the first months of life (0-2 months), which may be a result of protection by transplacental antibodies. The pattern of acquisition of rotavirus antibody was consistent with this age distribution of disease and with optimal age for vaccination. Thus, regional epidemiological characteristics of rotavirus infection may affect optimal performance of rotavirus vaccine (27).

Conclusion and Recommendation
The finding of this study indicates that 34.6% under five children of Addis Ababa with acute diarrhea are suffering from Rotavirus infection. Rotavirus infection affected all age groups of under five children, regardless of their socioeconomic background and there was no any single risk factor significantly associated with Rotavirus diarrhea. The result may show the need for continuous monitoring of viral enteropathogens for successful treatment and control of diarrhea. Long term community based surveys at regional and national levels are important to provide broader actual picture of the Rotavirus burden in Ethiopia.

Competing Interests
The authors declare that we have no competing interests.

Author's Contribution
MG, KD and AA designed study and participated in the preparation of this manuscript. MG participated in proposal development, sample transportation and testing. DM involved in drafting the manuscript and approved the final version for publication. SF and YM participated in pediatric stool sample transportation and examination. AD analyzed the data. All authors read and approved the final manuscript.
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Comparison of biophysical properties of depolymerase producing and non-producing phages of Klebsiella pneumoniae B5055

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Abstract
Background: Bacteriophages, also known as phages are viruses that infect bacteria. As a biological entity, it produces various types of enzymes.

Aims: The isolation and characterization of two virulent Bacteriophages were described on the basis of their ability to elaborate depolymerase enzyme against Klebsiella pneumoniae B5055.

Methods: Phage was isolated from sewage against host bacteria Klebsiella pneumoniae B5055 using standard protocols. Further biophysical characterization including electron microscopy and molecular characterization was done using standard procedures.

Results: Depolymerase enzyme producing and non-producing phage showed similar single step growth curve, adsorption rate, and head symmetry. SDS-PAGE pattern revealed bands with molecular weights ranging from \( \approx 129 \) to \( 18 \) kDa. Restriction analysis and RAPD fingerprinting indicated that both \( \Phi \text{DEP} \) and \( \Phi \text{NLP} \) phages possessed dsDNA with an approximate genome size of 40 kb and 49 kb, respectively and distinct entities. Both phages showed differences in physical stability conditions and moreover, \( \Phi \text{DEP} \) depolymerase enzyme activity showed several folds higher than \( \Phi \text{NLP} \) phage.

Conclusions: On the basis of these results, it is concluded that both the phages are different entities within the same family of Podoviridae differing in their ability to elaborate depolymerase enzyme. The production of depolymerase is therefore a specific feature of each individual phage.

Key words: depolymerase, phage, Klebsiella pneumoniae B5055, halo, capsular polysaccharide, plaque proper

Introduction
Many pathogenic bacteria including Klebsiella pneumoniae possess a polysaccharide capsule. This external structure protects the pathogens from the animal immune system and phage infection (1). Phages are bacterial viruses that over 95% of them described in the science belong to the Order Caudovirales (tailed phages). The three main families Siphoviridae, Myoviridae and Podoviridae are characterized by their unique tails appearance (2). It has been shown that some bacteriophages can digest these capsules due to their ability to produce depolymerase enzyme (3). It is a highly specific glycanohydrolase that randomly attacks the residue \( (\rightarrow 3)\)-\( \beta \)-D-Glucose p-(1\( \rightarrow 4 \))-\( \beta \)-D-Mannose p-(1\( \rightarrow 4 \))-\( \alpha \)-D-Glucose p-(1\( \rightarrow 3 \)) \( \alpha \)-D-Glucoronic acid p (linked to Mannose) linkage) of the capsular polysaccharides of K2 antigens of K pneumoniae (4). This ability of depolymerase has found many applications in recent years. From our laboratory we have reported the importance of depolymerase enzyme in treating the young and mature biofilm of K pneumoniae in conjunction with antibiotics (5). Another observation made by us indicates that depolymerase producing phages can be effectively employed for the therapeutic control of bacterial infections in experimental animals (6). On the contrary Pseudomonas phages which were non-producer of depolymerase enzyme were
ineffective when applied on burn wound infection in experimental mice (7), conferring the importance of depolymerase enzyme in the treatment process. Since phages which do not elaborate depolymerase enzyme also exist in nature hence the present study was carried out to see whether ΦNDP phage, which was selected based on absence of halo formation around the clear lytic plaque centre, has discernible activity difference in vitro. In addition, if there is additional difference exist between those phages with good depolymerase producing and non-depolymerase producing phages of K pneumoniae B5055 during initial isolation in terms of structural, genomic or biophysical attributes. In vitro data is always a prerequisite to be selected as candidate phage for therapeutic purposes.

Materials and Methods

Bacterial Strain and Bacteriophage Isolation
The strain of Klebsiella pneumoniae B5055 originally obtained from M. Trautman, Department of Medical Microbiology and Hygiene, Ulm, Germany and being maintained in the laboratory was used in the present study. The bacterial strain was grown aerobically in Luria broth at 37°C from -70°C glycerol stock. Prior to phage testing, the bacterium was subcultured at least twice in Luria broth. The bacterial growth in log phase (attaining an OD600 = 0.3 or ≈10⁶ CFU/ml in a freshly inoculated Luria broth medium) was used in all phage experiments unless otherwise mentioned. Bacteriophages active against K pneumoniae B5055 were isolated from sewage samples obtained from sewage treatment plant around Chandigarh by the enrichment method of Twest and Kropinski (8). After amplification, the presence of phages was checked by spotting the lysate supernatant on bacterial lawn. Titre of the phage preparation was estimated by agar overlay method and titre was expressed as plaque forming units per millilitre (pfu/ml). The purified phages were stocked in aliquots at 4°C and -60°C with 50% glycerol.

Selection of Phage
The technique described by Stirn and Freund-Molbert (9), as modified by Besser and coworkers (10) was followed to select a phage with halo formation to represent depolymerase producer. According to Adams and Park (11) and Stirn and Freund-Molbert (9), halos are due to the overproduction of free, enzymatically active "spikes" or "fibers" in addition to complete virus particles. A phage elaborating high amounts of depolymerase enzyme was selected based on the diameter of halo around plaque proper after overnight incubation at 37°C and termed as depolymerase producing phage (ΦNDP). Similarly, another phage with no halo formation around the plaque proper after 48 h was selected and termed as non-depolymerase producer (ΦNDP).

Adsorption time and Single-Step Growth Curve Experiment
Phage adsorption experiment was carried out according to the method of Adams (12) as followed in our laboratory by Kumari and colleagues (13). Briefly, to the K pneumoniae B5055 culture, phage suspension was added at a multiplicity of infection (MOI) of 0.1 and incubated at 37°C for 20 min. Aliquots (100 µl each) were taken at 4 min intervals (up to 20 min) and immediately centrifuged in a cooling centrifuge at 8000 x g for 1 min. The number of free infectious phage particles was calculated by phage titration employing double agar overlay technique. Single step growth curve of the phages was performed according to the method followed by Kumari and colleagues (13). Experiments were repeated on three different occasions and values depict the mean of independent observations.

Temperature, pH, and U.V. sensitivity
Temperature, pH and U.V. light stability, and temperature and pH optima of the phages were checked (14). The bacteriophage pH kinetics was studied for pH 3.5. This was selected based on the fact that all phages tested for pH stability were inactivated at pH 3.0 but not at pH 4.0. For all the phages exposed to pH 3.5, inactivation was enumerated at 37°C by withdrawing samples at different time intervals i.e. 0, 1, 3, 5, 8, 10, 15, 20, and 30 min. The samples were diluted in pH-adjusted and preincubated PBS buffer (0.10 M, pH 7.0). Phage titre was estimated by double agar overlay method. All phage counts were performed in duplicate at two different occasions. In all cases, results were reported as percentage inactivation against control phage held in PBS (0.10 M, pH 7.0) at 4°C. Stability of the phages to U.V. light exposure was evaluated by exposing the phage suspension in sterile Millipore water
(10⁴ pfu/ml) to U.V. bulb (U.V.-C, wave length of 254 nm) at a distance of 50 cm. Irradiated samples of 500 µl were collected at different time interval, i.e. 0, 5, 10, 15, 20, 25, 35, 45, 55, 70, 90, 100, 120, and 180 s, for immediate phage titration.

Organic Solvent (chloroform, ether and ethanol) sensitivity
The method described in Verma and his colleagues (14) was followed with little modification. Briefly, equal volume of bacteriophage (1 x 10⁶ pfu/ml) and appropriate absolute organic solvent (chloroform, ethanol, and diethyl ether) were mixed and incubated at room temperature with intermittent shaking. Mixtures were taken at 0, 5, 10, 15, 20, 30 and 60 min and phage titre in aqueous phase was estimated by soft agar overlay method using K pneumoniae B5055 as host. Equal volume of the bacteriophage and PBS buffer held in the same condition was used as control. Since these phages showed remarkable sensitivity to ethanol, the sample was collected at shorter intervals. Results are reported as percentage inactivation against control phages in PBS and repeated at least two times in duplicate.

Electron microscopy
High titre phage lysate dialysed against distilled water was concentrated by ultracentrifugation at 100,000 x g in a swinging rotor for 2 h (Beckman Coulter, Optima™ LE-80 Ultracentrifuge). The pellet was dropped on nitrocellulose coated grids (diameter, 3 mm; 300 meshes) and negatively stained with phosphotungstic acid (4% w/v) with pH adjusted to 7.0. Samples were viewed under an electron microscope (TEM; Hitachi 7500) at 80 kV at Sophisticated Analytic Instrumentation Facility (SAIF), Panjab University, Chandigarh, India. The phage size was determined from the average of 4 to 5 independent measurements.

Bacteriophage DNA restriction endonuclease patterns and RAPD-PCR
Nucleic acid of the phages was extracted and purified from lysates by proteinase K method followed by reuspension in Tris-EDTA buffer after ethanol precipitation as described elsewhere (15). Restriction enzyme digestion of isolated phage DNA was carried out following the instructions provided by suppliers. Restriction endonucleases, Hinf I, HaeIII, KpnI, EcoRI, Sau3AI, Apa I, Bgl I, Cla I, BamHI, or Hind III (all from GeNei™, Bangalore) was added to purified bacteriophage DNA. The restriction digests were separated on 0.8% agarose gel (HiMedia) in 1 x TAE buffer (40 mM Tris-acetate and 1 mM EDTA, pH 8.0) (HiMedia) containing 0.5 µg/ml of ethidium bromide (HiMedia) at 100 V for 1-2 h. Fragment sizes were determined from restriction enzyme digests of control (λ) phage.

Random Amplified Polymorphic DNA (RAPD)-PCR of bacteriophage DNA was carried out (13). Six 10-mer primers, Primer 1 (5’-GGTGCGGGGAA 3’), Primer 2 (5’-GTTTCGCTCC 3’), Primer 3 (5’-GTAGACCGGT 3’), Primer 4 (5’-AAGAGCCCGT 3’), Primer 5 (5’-AACCGCGCAAC 3’) and Primer 6 (5’-CCGTCAGCA 3’)(GeNei™, Bangalore) were used. Nucleic acid fragments (amplified or digested) were separated by electrophoresis on 2% agarose gel.

SDS-PAGE of bacteriophage proteins
Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out based on the standard method of Laemmli (16) and as adopted by Sambrook and co-workers (15) for separating structural proteins of the phages. High phage titre lysates were concentrated using Pellicon™ XL (Molecular Weight Cut-Off ‘MWCO’ = 5 kDa) device (Millipore™, India). Protein molecular weight marker (GeNei™, Bangalore) was used for size determination of proteins and separation of phage proteins was carried out in 12% polyacrylamide gel. Protein bands were visualized on the gel following silver staining.

Phage fragmentation for depolymerases production
High titre phage lysate (10¹¹ to 10¹² pfu/ml) served as crude enzyme source. Bacterial cell and cell debris was removed by centrifugation (8,000 x g, for 10 min, at 4°C) and supernatant was treated with chloroform (1-2% v/v) and again centrifuged (10,000 x g, for 10 min, at 4°C). The dialysed lysate was handled and treated (17,18) to fragment the phages by adjusting its pH to 3.5 for 20 min and then brought back to pH 7.0 using aqueous Tris. Titre of phage (pfu/ml) was determined before and after acid treatment to assess the level over infective phages in the preparation. The enzyme activity and total protein was also determined as mentioned below.
Determination of capsule depolymerase activity

The capsule depolymerase activity was determined by spot assay according to the method of Adams and Park (12) as modified later by Sutherland and Wilkinson (19), and quantitatively measured by the method of Rondel and Morgan (20). In the former case, the titre of highest dilution giving dissolution of capsular polysaccharide (CPS) of K pneumoniae B5055 was recorded as a reciprocal value of the enzyme activity. In the latter method, quantity of liberated reducing sugar was spectrophotometrically estimated due to the activity of phage depolymerase on CPS of the bacterium. One unit of depolymerase activity was defined as the amount of enzyme that catalyzes the reaction of 1 nmol of CPS per min per ml of enzyme at specified conditions. Lowry method was used to measure protein concentration (21). Galactosamine and bovine serum albumin were used as standards for CPS and protein estimations respectively.

Extraction of Capsular Exopolysaccharide of K pneumoniae B5055

The bacterial exopolysaccharide was isolated from K pneumoniae B5055 (22). Dried precipitated CPS was prepared and dissolved in sterile distilled water. The CPS was stored at -20°C until used. Polysaccharide estimation was done as described by Dubios and the team (23) using glucose as standard.

Statistical Analysis

Statistical analysis of the data was entered and done using Origin Pro 8 SRO 2007 Program Software. All experiments on the two phages were repeatedly performed at least on two or three different circumstances and, in all cases, the mean values of independent observations were used in the analysis. The difference of means of the two phages feature was compared. P value of less than 0.05 was considered statistically significant.

Results

Bacteriophage isolation

In the present study, K pneumoniae B5055 was used as an indicator strain for the isolation of lytic bacteriophages from sewage samples collected from Chandigarh city in India. Thirteen bacteriophages specific for encapsulated K pneumoniae B5055 were isolated. These specific bacteriophages demonstrated plaque proper (lytic clear centre) with or without halo (translucent clearance around the plaque proper) formation. Depending on this observation, two phages were selected: a phage that exhibited a maximal halo zone compared to its clear plaque proper and labelled as depolymerase producing phage (ΦDEP), and another that produced plaque proper with no halo as non-depolymerase producing phage (ΦNDP). The plaque proper of ΦDEP was significantly smaller than ΦNDP phages (p = 0.00345) after 24 h of plaque assay. In the former case, the plaque typically formed bigger double halo formation around plaque proper (6.2 ± 0.3 mm) at 37°C in 24 h (Fig. 1a). The halo formation around clear center became bigger upon further incubation (Fig. 1c & 1d). In case of ΦNDP, the diameter of plaque proper was 2.8 to 4.0 mm after 24 h of incubation with no halo formation. Both phages were selected, purified, and amplified for further characterization and comparison of their biophysical properties.
Phage growth characteristics

Results presented in Fig. 2 show the adsorption rate of phage particles onto bacterial cells. A great share of ΦDEP phage particles (about 61%) were adsorbed onto host cells within the first 4 min; 91% in 12 min, and 96% in 16 min. On the other hand in case of ΦNDP phage, about 54% of the particles were adsorbed onto the host cells within four minutes and it became 87% in 12 min and 91% in 16 min. Statistically no difference was observed in the rate of adsorption for both the phages (p = 0.633). Single (one) step growth curve experiment was done to identify the different phases of growth for both the phage. ΦDEP and ΦNDP phages showed similar eclipse period of 10 min and latent period of about 20 min. On the other hand, ΦDEP has a burst size of 118±2.0 pfu per bacterial cell as calculated from the phage counts while in case of ΦNDP the burst size was 123±1.2 pfu per infected bacterial cell.

Temperature, pH, and U.V. stability

Both the phages were stable over a period of an hour when exposed to temperature range of 0 to 40°C. However, at 56°C, about 17% and 23% reduction in phage activity was observed for ΦDEP and ΦNDP, respectively. A complete inactivation of ΦDEP phage was observed at 60°C after 1 h of exposure, while about 67% of ΦNDP phage particles were not inactivated (Fig. 3a). The optimum temperature for activity of both the phages at pH 7.0 was found to be between 37 to 40°C even though slight change in the temperature did not alter infectivity of the phages significantly. In addition, both the phages were found stable without palpable loss of titre at -60°C after one year (data not shown). The test regarding pH stability of the phages held at room temperature for 1 h showed that ΦDEP and ΦNDP phages lost their infectivity completely at pH 2, 3 or 13 (Fig. 3b). It was observed that ΦDEP was more stable at alkaline
pH than ΦNDP. The latter showed stability around neutral pH and showed decline in its titre when exposed to harsh acidic or alkaline pH. The optimum pH for ΦDEP phage activity was observed at pH 8.0 unlike ΦNDP (pH 6.0) at 37°C (data not shown).

Sensitivity of ΦDEP and ΦNDP to U.V. light was also evaluated by exposing each phage to U.V. light for a period of 3 min. The results showed complete inactivation of ΦDEP bacteriophage after 45 s of U.V. exposure while ΦNDP got inactivated after 120 s. Hence, ΦNDP was comparatively more tolerant to U.V. than ΦDEP phage. However, in both cases, about 99% of phages got inactivated in the first 15 s of U.V. exposure.

and ΦNDP phages after one hour of exposure to various temperature values. Surviving phages was determined using plaque assay. (b) Shows stability of phages (≈1 × 10⁶ pfu/ml) followed after exposure to various pH values at room temperature for 1 h.

Organic solvent sensitivity

Organic solvents including chloroform, diethyl ether and absolute ethanol were used to check ΦDEP and ΦNDP phage sensitivity. No effect on phage activity was seen after 1 h of incubation with chloroform and diethyl ether. However, after absolute ethanol treatment for an hour, activity of both the phages was completely destroyed. Then phage activity was followed over fraction of minutes after exposure to ethanol and in both phages most activity was lost within the first 15 seconds of exposure.

Restriction endonuclease and RAPD PCR patterns and sizing

The genomic DNA of each phage was isolated and electrophoresed on 0.8% agarose gel to check their purity and concentration. Restriction enzymes including HinfI, HaeIII, KpnI, EcoRI, Sau3AI, Apal, BglII, CiaI, BamHI, and HindIII, were used. The phage DNA of both the phages was not susceptible to restriction digestion with any of the following enzymes: KpnI, EcoRI, Apal, BamHI, and HindIII. In addition, ΦNDP DNA was not digested with BglII and CiaI (Fig 4). From the results of digested DNA, it was concluded that both phages possessed double stranded DNA and the genome size of ΦDEP and ΦNDP was estimated to be 40kb and 49kb, respectively.

Fig. 3. Stability of phages at different temperature and pH values. (a) Shows surviving active ΦDEP

Fig. 4. Restriction enzyme digestion pattern of ΦDEP (a) and ΦNDP (b) phage following agarose gel electrophoresis. On the (a) gel, Lane 1, ΦDEP DNA digested with Sau3AI; Lane 2, ΦDEP DNA digested with HinfI; Lane 3, ΦDEP DNA digested with HaeIII; Lane 4, ΦDEP DNA digested with CiaI; Lane 5,
ΦDEP DNA digested with BglII; Lane 6, ΦDEP DNA incubated with EcoRI but not digested. On the (b) gel, Lane 1, ΦNDP DNA digested with HaeIII; Lane 2, ΦNDP DNA digested with HindIII; Lane 3, ΦNDP DNA digested with Sau3AI; Lane 4, ΦNDP DNA incubated with EcoRI but not digested; and Lane 5, ΦNDP DNA incubated with BamHI but not digested. On both 4 (a) and (b) gel, Lanes M1 and M2 were 1 kb ladder and lambda phage DNA digested with HindII restriction, respectively as reference marker; Lane C: Control of the undigested ΦDEP and ΦNDP phage DNA respectively.

RAPD PCR was performed with the DNA samples isolated from the two phages. Six different primers were used in random amplification, out of which five of them provided bands for ΦDEP phages; but only Primer 3 produced band for ΦNDP phage DNA. As a result of these, both phages exhibited different patterns showing that they are genetically unique (Fig.5). Electron microscopy
Both the phages were observed under transmission electron microscope (TEM) after negative staining with phosphotungstic acid (4% w/v, pH 7.2). Both showed an icosahedral capsid and a short tail. The diameter of the capsid of ΦDEP was 44 ± 0.4 nm while ΦNDP was 44± 1.0 nm. ΦDEP phage tail was shorter (10 to 12 nm) than ΦNDP phage which had a 15.6 to 15.9 nm long tail. These findings allow their grouping in the order Caudovirales and family Podoviridae (Bradley group C) since the phages having a small non-contractile tail belong to this family (Fig. 6).

Depolymerase enzyme assay
Spotting of a twofold serial dilution of crude enzyme was carried out on a matured lawn of K pneumoniae B5055 and the reciprocal of the highest dilution that showed a perceptible dissolution of the lawn was taken as the activity of

with HindIII restriction as a reference marker. Amplified fragments were separated by electrophoresis on 2.0% agarose gel.

Fig. 6. TEM appearance of ΦDEP (a) and ΦNDP (b) virions upon negative staining with phosphotungstic acid.

Phage structural protein gel electrophoresis
SDS-PAGE showed six major protein bands and about nine minor protein bands according to their relative mobility, with molecular weights ranging from 129 to 18 kDa (Fig. 7). Both phages showed similar major bands with little variation in the minor bands. The major bands molecular weight ranged from 59.3 kDa to 23 kDa (59.3, 41.1, 38.5, 31.2, 29.6, and 23.4 kDa) on SDS PAGE.

Depolymerase enzyme assay
Spotting of a twofold serial dilution of crude enzyme was carried out on a matured lawn of K pneumoniae B5055 and the reciprocal of the highest dilution that showed a perceptible dissolution of the lawn was taken as the activity of
depolymerase. In ΦDEP, the activity of the depolymerase was 320 while in ΦNDP it was about 10 which had equal plaque titre per millilitre. Quantitative depolymerase enzyme activity of equal phage particle titre was also determined as described in material and methods for both the phages. There was a palpable difference between two of the phages depolymerase enzyme produced (p<0.0137). The crude high titre ΦDEP lysate had 36 U/ml of enzyme activity compared to 6.3 U/ml of ΦNDP. On the basis of this, ΦDEP has nearly six times higher depolymerase activity than ΦNDP phage.

Discussion
Depolymerase enzyme helps the bacteriophage in reaching close to the bacterial surface in order to establish the infection in the cell. Therefore, we focused on the isolation and characterization of the bacterial virus depolymerase against the host bacterium Klebsiella pneumoniae B5055, having potential use for in vivo application. Many bacteriophages capable of infecting encapsulated bacteria, like K. pneumoniae, carry capsular (exo-) polysaccharide degrading enzymatic activity, that is easily spotted on the basis of various sizes of halo around the clear lysed plaque proper (11). Based on this knowledge, thirteen phages from the environmental sewage samples were isolated and the one giving maximum halo around plaque proper was selected (ΦDEP). For comparative biophysical properties another phage was also selected which produced no halo around (ΦNDP) plaque proper.

Initial characterization of ΦDEP and ΦNDP was done based on the basis of plaque morphology. In case of ΦDEP, although the diameter of double halo zone plus the plaque proper was significantly greater than ΦNDP plaque size, the later produced a larger plaque proper than the former. This may be as a result of its comparatively higher burst size per infected bacterial cells compared to ΦDEP. The halo zone of ΦDEP surrounding phage proper extended upon further incubation; though phage replication somehow stopped in less than 24 h indicating that phage sub organelles in soluble form which diffused through the medium were responsible for increasing the size of halo. While in ΦNDP, no such increase in plaque proper or halo was seen even upon extended incubation.

Both methods of quantitative determination of depolymerase activity showed that ΦDEP phage possesses superior activity of the enzyme that was many times more than the units found for ΦNDP phage. On the basis of this, unlike ΦDEP phage, which elaborated excessive depolymerase freely diffusable into the medium, further evaluation of ΦNDP phage showed minute levels of depolymerase enzyme activity. This finding may be due to the existence of the enzyme associated firmly with the phage particle (11). Earlier reports (10, 24) indicated that ‘spikes’ observed on the tails of certain phage particles are in fact phage bound form of depolymerase and their appearance in medium may be due to its overproduction and release, which is under the control of phage genome. Therefore it is imperative to assume that the way both phages possess and use their decapsulating enzyme is the result of difference in their architecture to hold the enzyme and consequently the result of their difference in expressing from genome.

Both phages were selected, purified, and amplified for further biophysical comparison. Probably the most important criteria for characterizing phages are based on their distinct morphological and genetic characteristics. Transmission electron microscopy and genetic characterization of both ΦDEP and ΦNDP phages showed icosahedral head symmetry with pentagonal outlining and with short non contractile tails. Both phages possessed double stranded DNA and belonged to Podoviridae family with a Bradley morphological group C appearance (2). RAPD-PCR and restriction enzyme digestion confirmed differences among these two phages.

The protein profile of a bacteriophage is very unique, as it represents the products of the viral genome. The more related viruses share major proteins, making this as a good marker to judge their relatedness. Both ΦDEP and ΦNDP showed proteins with similar molecular weights ranging from ~129 to 18 kDa (Fig. 7). There were major bands with little variation in the minor proteins. The molecular weight of the isolated phage DNA corresponded with earlier reported molecular weight of phages infecting enterobacteria such as Lambda, and T3/T7 (2). Based on their genome size it appears that these two phages are more close to Lambda or T3/T7 like phage (25). However, on the basis of the results of transmission electron microscopy, the morphology of ΦDEP and ΦNDP phages was not similar to that of Lambda phage and therefore
these cannot be classified as Lambda-like. The speculative similarity between the unknown phages in this study and T7 phage on the basis of morphological observation under electron microscope confirms that the phages are members of Podoviridae family. Bradley proposed phage classification scheme that depend on nucleic acid type and gross morphology, where our both phages were morpho-types C.

Bacteriophage ϕDEP and ϕNDP when exposed to various organic solvents were found to be stable in presence of chloroform and ether. Hence, it can be deduced that they are devoid of lipid components in their structure. Moreover, chloroform can be used as an agent to prevent bacterial contamination or used in their isolation from the environmental samples. Both phages were also found to be stable over a wide range of pH (4–11) and temperature when exposed for 1 h. However, there were differences in their stability against certain physical parameters. For example, differences in their optimum pH values were noticed. ϕDEP phage best acted at pH of 8.0 unlike ϕNDP which was more active at pH 6.0 at 37°C. On another parameter, ϕNDP phage showed better tolerance to U.V. light than ϕDEP (p = 0.0074). These results indicated that since these phages were stable at diverse conditions hence these can be considered suitable for different applications.

One of rich source of enzymes degrading bacterial capsular exopolysaccharides has been proved to be bacteriophages (3). However, much of the study to phage depolymerase enzyme types were not dealt with suitably in the current century as much as there had been in the 1950s to 1980s. In the case of ϕDEP phage lysates, unlike ϕNDP, it normally contained further amounts of the same enzyme in soluble form. As a result of this, ϕDEP phages may have superior application than ϕNDP in degrading the pathogenicity of encapsulated bacteria such as Klebsiella pneumoniae, as in effective disruption of biofilm polymeric matrixes (26), as a system used in elucidation of the structural components of polysaccharides and other application (3).

Conclusion
On the basis of these results it can be concluded that both the phages were different although they were related to each other. Moreover, they were capable of efficiently lysing encapsulated K pneumoniae B5055 differing only in their ability to elaborate extracellular depolymerase enzyme. The production of extracellular depolymerase is therefore a specific feature of each individual phage.

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Abbreviations: (ϕDEP), depolymerase producing phage; (ϕNDP), non-depolymerase producing phage

REFERENCES
Assessment of clinical Laboratory service utilization in the outpatient department at University of Gondar Hospital, Northwest Ethiopia

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Abstract
Background: Clinical laboratory service is the backbone of health care facilities which gives valuable information for patient care. Distribution of diagnostic clinical laboratory tests are different in type and quantity of test at different health facilities such as health center, district hospitals, referral hospitals and specialized hospitals for many of the preventable diseases in Ethiopia. The main aim of the present study was to assess clinical laboratory service utilization in the outpatient department at University of Gondar Hospital laboratory.

Methods: A one year laboratory service data were collected from a daily registration book retrospectively for analysis of outpatient service utilization of University of Gondar hospital laboratory.

Result: A total of 59,605 outpatients visited the laboratory from July 2013 to June 2014. About 59.7% of the outpatients were from outside Gondar Town. In the study period, 31.1% were not charged for the service due to poverty certificate. Children under 15 years old accounted 7.8% of service consumers. A total of 235,653 tests were done in hospital laboratory during one year study period. Of these tests, urinalysis tests (53,125(25.6%)) and clinical chemistry tests (52,497(25.5%)) were frequently requested tests. Majority of the tests (26.7%) were done in the 3rd quarter of the year especially in the month of March. Tuesday and Friday were the days of the week in which the peak number of outpatients and tests were recorded.

Conclusion: Urinalysis and clinical chemistry tests were the most frequently utilized clinical laboratory tests. The utilization of clinical Laboratory service in the outpatient department at University of Gondar hospital was not equally distributed throughout the week as well as months of the year. Therefore, University of Gondar hospital laboratory may give emphasis the seasonal variation of clinical Laboratory service utilization and section based workloads.

Key words: Clinical laboratory service, Ethiopia, Outpatient

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diseases. Therefore, health facility planning must consider the clinical laboratory service basic supply requirements (3). Laboratory testing is performed 24 hours a day, seven days a week. The majority of tests are performed on site. Certain tests are referred to designated and approved reference laboratories. Testing is performed by skilled medical laboratory technologists and other trained professionals using the available instruments in the facility. The rapid turnaround time of tests in the laboratory provides physicians to treat their patients in a timely fashion. Moreover, in-service training and continuing education programs on a regular basis for laboratory professionals have paramount importance to maintain and improve their laboratory professionals’ skills.

The quantity and quality of health care in Ethiopia is extremely low due to regular shortages of laboratory services. The rural area which consists 85% of the population in the country has no adequate infrastructure and are far from urban health facilities so that it imposes difficulty to deliver health care to hard-to-reach groups. So decentralization of health services depends on basic health services for the rural population and should include the parallel development and strengthening of laboratory units to enhance the diagnostic, therapeutic and surveillance activities of the health institutions (2). Such type of planning consideration will, however, require information on expected customer patterns. One of the best information for this planning can be obtained from studies investigating diagnostic laboratory service consumption at the health facility laboratories especially for outpatient needs. The main goal of this study was to assess the pattern of laboratory service consumption at University of Gondar hospital, Northwest Ethiopia.

Methods
This study was conducted at University of Gondar hospital laboratory using daily registration book from July 2013 to June 2014. Gondar is located 739 km far from Addis Ababa to the Northwest of Ethiopia with latitude and longitude of 12°35’N 37°26’E with an elevation of 2,133 meters above sea level. It has a total population of 207,044 of whom 96,120 are men and 106,924 women (4). University of Gondar hospital currently has 500 beds, and acts as the referral centre for the nearby district hospitals and serves for a population of

more than six million across the region as well as to the nearby regions. University of Gondar hospital laboratory was established in 1954. It gives diagnostic service for ward and outpatient departments and as a basic training center for under graduate as well as post graduate students of clinical laboratory science in the school. It has seven sections; the sections and the respective tests done in them are Hematology (CBC, ESR, Coagulation time, peripheral morphology, hem parasite, semen analysis, skin snip for microfilaria), clinical chemistry (Glucose, liver function tests, renal function tests, Hormone tests and electrolyte), parasitology (direct stool microscopy and concentration technique, occult blood and stool antigen tests), serology (widal, WWF, VDRL, C-TP, ASO, HCV, HBsAg, TPHA, ANA, anti Toxoplasmosis), bacteriology (AFB, Grams stain, wet mount, KOH wet mount, culture from body fluids), and urinalysis (urine biochemical and microscopy tests, HCG). These laboratories were included in this study. All patients who came from the outpatient department with requests of laboratory tests requested by physicians of the hospital were documented and socio-demographic characteristics such as age, sex, type of test requested, address of patient and the price of the tests as well as paying status such as paid, free, credit were included in the study.

Data collection tools and procedures
Using a structured checklist, information on sex, age, address, types of tests requested and paying status were assessed from the daily registration book. The data was entered, cleaned and analyzed using SPSS statistical software version 20. Then, study findings were presented using tables, and figures. Proportions for categorical variables were compared using chi-square test. A p-value of less than 0.05 was considered as statistically significant.

Ethical considerations
Ethical clearance was obtained from University of Gondar, School of Biomedical and Laboratory Sciences before commencement of this study. Data taken from each study subjects were coded and results obtained from each study subjects were kept always confidential.
Result
A total of 59,605 patients visited the clinical laboratory services from July 2013 to June 2014. About 34,929 (58.6%) of the patients were females and 24,676 (41.4%) were males. The mean age of the study participants was 35. Patients in the age group of 15-49 years constituted 56.1% of the customers. Children under five years were 3.5% and less than 15 years old were 7.8%. Majority of the study subjects (59.7%) were from outside Gondar Town and the remaining 40.3% were from Gondar Town (Table 1).

Table 1: Socio-demographic Characteristic of clinical Laboratory service utilization in the outpatient department at University of Gondar Hospital, Northwest Ethiopia from July 2013 to June 2014

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male N (%)</th>
<th>Female N (%)</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>1200(56.5)</td>
<td>924(43.5)</td>
<td>2124(3.6)</td>
</tr>
<tr>
<td>6-15</td>
<td>1350(53.2)</td>
<td>1189(46.8)</td>
<td>2539(4.3)</td>
</tr>
<tr>
<td>16-49</td>
<td>15473(38)</td>
<td>25250(62)</td>
<td>40723(68.3)</td>
</tr>
<tr>
<td>≥50</td>
<td>7350(51.7)</td>
<td>6868(48.3)</td>
<td>14218(23.8)</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outside Gondar</td>
<td>20987(59.0)</td>
<td>14597(41.0)</td>
<td>35584(59.7)</td>
</tr>
<tr>
<td>Inside Gondar</td>
<td>11390(47.0)</td>
<td>1227153.0</td>
<td>24021(40.3)</td>
</tr>
<tr>
<td>Paying status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paid</td>
<td>19981(54.3)</td>
<td>16828(45.7)</td>
<td>36809(61.7)</td>
</tr>
<tr>
<td>Free</td>
<td>7234(38.9)</td>
<td>11345(61.1)</td>
<td>18579(31.2)</td>
</tr>
<tr>
<td>Credit</td>
<td>3100(73.5)</td>
<td>111726.5</td>
<td>4217(7.1)</td>
</tr>
<tr>
<td>Total</td>
<td>24675(41.4)</td>
<td>34929(58.6)</td>
<td>59605(100)</td>
</tr>
</tbody>
</table>

About a total of 235,653 tests were done in hospital laboratory during this one year study period. Of these tests, 53,125(25.6%), 52, 497(25.5%), 43,321(16.4%), 27,942(11.6%), 17,174(7.3%), 13,643(5.9%) and 7,950(3.3%) were urinalysis, clinical chemistry, hematology, EOPD, serology, parasitology and bacteriology tests respectively (Table 2). Majority of the tests (26.7%) were done in the 3rd quarter of the year especially the peak month was March (Fig1 and Table 2). Tuesday and Friday were the peak days of the weeks which accounted for more than 50% of the week’s workload. About 50% of the outpatients were registered for more than four tests whereas 5% were registered for only single tests and more than five types of tests were frequently requested for more than 50 age groups of the customers in both sexes.

![Fig.1. Address and number of patients attending University of Gondar hospital laboratory from July 2013 – June 2014](image-url)
Table 2: Number of tests done in each section University of Gondar hospital laboratory from July 2013 – June 2014

<table>
<thead>
<tr>
<th>Month</th>
<th>Serology</th>
<th>Para</th>
<th>Hema</th>
<th>UA</th>
<th>Chem</th>
<th>Bact</th>
<th>EOPD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>JULY</td>
<td>1387</td>
<td>848</td>
<td>3193</td>
<td>4226</td>
<td>4286</td>
<td>766</td>
<td>1836</td>
<td>16542</td>
</tr>
<tr>
<td>AUG</td>
<td>1322</td>
<td>722</td>
<td>2709</td>
<td>4238</td>
<td>3968</td>
<td>893</td>
<td>2177</td>
<td>16029</td>
</tr>
<tr>
<td>SEP</td>
<td>1061</td>
<td>1024</td>
<td>3273</td>
<td>4801</td>
<td>4262</td>
<td>1061</td>
<td>2225</td>
<td>17707</td>
</tr>
<tr>
<td>OCT</td>
<td>1474</td>
<td>896</td>
<td>3334</td>
<td>4342</td>
<td>3943</td>
<td>408</td>
<td>2132</td>
<td>16529</td>
</tr>
<tr>
<td>NOV</td>
<td>1396</td>
<td>1024</td>
<td>3496</td>
<td>4663</td>
<td>5106</td>
<td>583</td>
<td>2501</td>
<td>18769</td>
</tr>
<tr>
<td>DEC</td>
<td>1441</td>
<td>1001</td>
<td>3529</td>
<td>5026</td>
<td>4890</td>
<td>512</td>
<td>1881</td>
<td>18280</td>
</tr>
<tr>
<td>JAN</td>
<td>1533</td>
<td>940</td>
<td>3450</td>
<td>4806</td>
<td>4980</td>
<td>589</td>
<td>2720</td>
<td>19018</td>
</tr>
<tr>
<td>FEB</td>
<td>1411</td>
<td>1394</td>
<td>4132</td>
<td>4791</td>
<td>6240</td>
<td>570</td>
<td>2822</td>
<td>21360</td>
</tr>
<tr>
<td>MAR</td>
<td>1846</td>
<td>1891</td>
<td>4342</td>
<td>8864</td>
<td>7327</td>
<td>818</td>
<td>2198</td>
<td>27286</td>
</tr>
<tr>
<td>APP</td>
<td>1429</td>
<td>1577</td>
<td>3906</td>
<td>6401</td>
<td>5723</td>
<td>520</td>
<td>2668</td>
<td>22224</td>
</tr>
<tr>
<td>MAY</td>
<td>1443</td>
<td>1373</td>
<td>4347</td>
<td>5541</td>
<td>6564</td>
<td>568</td>
<td>2621</td>
<td>22457</td>
</tr>
<tr>
<td>JUNE</td>
<td>1431</td>
<td>1153</td>
<td>3610</td>
<td>5427</td>
<td>5208</td>
<td>662</td>
<td>2161</td>
<td>19652</td>
</tr>
<tr>
<td>Total</td>
<td>17174</td>
<td>13843</td>
<td>43321</td>
<td>63126</td>
<td>62497</td>
<td>7950</td>
<td>27942</td>
<td>235853</td>
</tr>
</tbody>
</table>

Key: EOP- Emergency outpatient department, Bact- Bacteriology, Chem- chemistry, UA- Urinalysis, Hema- hematology, Para- parasitology, Sero- Serology

The price of each clinical laboratory tests were adjusted by the hospital top management from the least price 5.00 Birr for Hematocrit/hemoglobin, 12 Birr for stool direct microscopy, 25 Birr for serum H. pylori, 30 Birr for Coagulation, 30 Birr for culture, 33 Birr for CBC, 55 Birr for stool antigen tests to highest price 90.00 Birr for hormone tests. The paying status of study participants with certificate of poverty and credit were 31.2% (18, 579/59,605), and 7.1% (4,217/59,605), respectively who were not charged money for getting service. Somewhat increased number of children frequency were seen in the first and fourth quarter of the study period whereas more adults were observed in the third quarter of the year.

Discussion
This study indicates that most of the benefited patients in this hospital laboratory service were adult age groups (91.6%). This is almost similar with studies which were done in Ethiopia (5, 6, 7, 8). Even though children were 45% of the general population of Ethiopia and more vulnerable for morbidity and mortality than adults, their contributions in seeking laboratory service in this health facility were lower than adults. This is also in line with the study previously conducted in Ethiopia (5, 7, 8). This may be due to economical or general dependency of this segment of the population on their guardians’ awareness or commitment. The other reason may be due to transportation and economical problems of the rural population in the region. For these reasons, populations living nearby to health facilities are more advantageous than those living far away. Economical wellbeing’s of the population, infrastructures like accessible and cheap transportation is a good opportunity for the health service utilization of the population (5, 6, 7, 8, 9). For this evidence clinical laboratory service consumers who were in the nearby Kebeles of Gondar town visited the service repeatedly but patients from remote Kebeles and outside Gondar
came to the hospital after harvesting seasons like the month March (10,11). The workload of the hospital laboratory showed that there was a patient load difference in the months of the study year. Patients coming in the month of March were much higher than the other months of the year. Especially many patients coming from outside Gondar visited the service in the 3rd quarter especially in the month of March. This may be due to the delayance of tests done in the hospital laboratory so that the rural area outpatients may prefer the 3rd quarter of the year since they are relatively free of farming activities. Mostly requested tests were routine tests like clinical chemistry and urinalysis tests which accounted for 51.1% of the tests done in the study period. This is almost similar with studies which were conducted in Ethiopia (5, 7, 8). Variations in workload of laboratory tests were seen among days of the week as well as months of the year.

Conclusion
Urinalysis and clinical chemistry tests were frequently requested clinical laboratory tests. Female sex, residents outside Gondar town and patients within productive age group predominantly utilized clinical laboratory services. Moreover, clinical laboratory service utilization in the outpatient department at University of Gondar Hospital was not equally distributed throughout the week as well as months of the year especially the rural population service utilization is seasonal due to the working condition of the farm land and difficulty in infrastructure.

Recommendation
Decentralization of health services by focusing on basic health care for the rural population must include parallel development of a network of laboratory units. Planning together with laboratory professionals, budgeting the laboratory service separately and close follow-up for the proper utilization of resources especially for the month of March which have peak number of patients in the year which is near to the end of the budget year is essential. University of Gondar hospital laboratory should do referral tests and supervise service provided by the nearby health facilities since it is a training center for medical laboratory technology students.

Competing interest
The authors declare that we have no conflict of interests.

Authors' contribution
Fikir A. wrote the manuscript, participated in data collection and analyzed the data. Ayenew A., Mulugeta M., Bamlaku E. revised the manuscript, participated in data collection and analysis. All authors participated in the statistical analysis, editing the manuscript and approved the final manuscript.

Acknowledgement
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Reference


Diagnostic laboratory service availability in Ethiopia: Health Facility Assessment

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Abstract
Background: The survey is designed to generate a set of indicators on key inputs and outputs of the health system, which can be used to measure progress in health system strengthening. The aim of the survey was to assess the availability and preparedness of health facilities in Ethiopia to provide quality laboratory services.

Method: The assessment was part of the 2014 Ethiopia Service Provision Assessment Plus (ESPA+) Survey. A total of 1,327 health facilities (hospitals, selected health centers, private clinics, and health posts) were assessed. Of these, 1,165 (88%) facilities were included in this study.

Results: Fifty seven percent of the facilities excluding health posts in Ethiopia had laboratory diagnostic services. HIV diagnostic test (59%) is the most commonly provided basic laboratory services. Liver or renal function tests were the least (7%) provided laboratory services in Ethiopia. Twenty percent of the facilities excluding health posts provided haemoglobin tests. Facilities managed by non-governmental organisations (40%) offered haemoglobin test. Facilities in urban areas (37%) offered greater proportion of the services compared to facilities located in rural areas (13%). Commonly provided laboratory services in Ethiopian facilities (hospitals, higher clinics, and medium clinics) were CSF/body fluid analysis (45%), and stool microscopy (42%). Majority of referral hospitals and general hospitals (94%), and most of higher clinics (88%) offered Gram's stain test. The least available advanced diagnostic services in Ethiopian facilities excluding health posts include serum electrolyte test (10%), full blood count with differential (10%), blood typing and cross matching (3%), CD4 count (3%), syphilis serology (3%), TB culture (1%), and TB rapid tests (<1%). CD4 count was performed in less than 10% of the facilities in all regions. Serum electrolyte and full blood count tests were done one in four facilities found in Addis Ababa, Harari, and Dire Dawa. Fifty three percent of the health posts provide malaria diagnostic tests and fourteen percent of the health posts offer HIV diagnostic test in Ethiopia.

Conclusion: The assessment indicated gaps in most of the laboratory services which could considerably impact quality and accessibility of diagnostic services. This suggests that there is urgent need to improve the capacity of diagnostic laboratories to offer quality services across health facilities in the country.

Key words: Service provision, laboratory, Ethiopia

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Background
Ethiopia’s Growth and Transformation Plan (GTP) has been designed to maintain rapid and broad-based economic growth and eventually to end poverty (1). The Health Sector Development Program (HSDP) is a key component of the GTP. Its primary objective is to improve the health of the population through promotion of preventive, curative and rehabilitative health services by...
improving access to affordable health services; and improving the quality of health services (2). Ethiopia has a three-tier health care delivery system: level one is a Woreda/District health system comprised of a primary hospital (serves 60,000 -100,000 population), health centres (one health centre serves 15, 000-25,000 population) and their satellite Health Posts (1/3,000-5,000 population) connected to each other by a referral system. The primary hospital, health centre and health posts form a Primary Health Care Unit (PHCU). Level two is a General Hospital serving 1.0-1.5 million population and level three is a Specialised Hospital serving a population of 3.5-5.0 million (2).

The decentralization of power to regional governments has resulted in largely shifting the decision making for public service delivery from the centre to being under the authority of the regions and down to the district level. Offices at different levels from the Federal Ministry of Health to Regional Health Bureaus (RHBs) and Woreda Health Offices share in decision making processes, powers, duties and responsibilities. The Ministry and the RHBs focus more on policy matters and technical support while Woreda Health Offices manage and coordinate the operation of the district health system under their jurisdiction (2).

Rapid expansion of private for profit and non-governmental organization (NGO) sectors are augmenting the public/private/NGO partnership for health and boosting health service coverage and utilization (2).

The government has taken a wide range of measures to improve the health status of the population. A number of health sector policies and programs have been developed and aggressively implemented. National health policy was adopted in the early 1990s (3) and strategies such as nutrition strategy, child survival strategy and infant and young child feeding strategies were endorsed subsequently. A number of innovative programs and interventions have been developed and implemented to translate the policies and strategies into action. The HSDPs and the health extension programs can be considered as the centre pieces in this accord. Changes in health care governance and health system management have been introduced. Decentralization in health care governance and management has been adopted. Relentless efforts have been made in expanding health facilities, human resource development and health care financing (3).

The major health problems of the country remain largely preventable communicable diseases, reproductive health related problems and nutritional disorders. Despite major progress, have been made to improve the health status of the population in the last two decades, Ethiopia’s population still face a high rate of morbidity and mortality and the health status remains relatively poor. Figures on vital health indicators from EDHS 2011 show a life expectancy of 54 years (53.4 years for male and 55.4 for female), and an IMR of 59/1000 (4).

There are multiple components that will influence this: available infrastructure, staff deployment and presence, and availability and quality of laboratory services provided. The World Health Organization (WHO) recognizes quality laboratory services as key to improving global health and reaching Millennium Development Goals. Strengthening the breadth of laboratory services accessible to clients, and ensuring that results are accurate, reliable, reproducible and rapid enough to be useful is crucial to improved health outcomes.

Although routine reporting will contribute to this understanding, at this stage of the implementation of routine reporting, national surveys are required to further complement the available routine reporting.

Objective

The major objective of the survey is to assess the availability and preparedness of health facilities in Ethiopia to provide quality laboratory services.

Methodology

Study area and design

Ethiopia has a federal structure composed of nine Regional States and two City Administrations. This study is a cross-sectional study, which combines MEASURE DHS SPA, World Health Organization’s service Availability and Readiness Assessment (SARA) and the World Bank’s Service Delivery Indicator (SDI).
Data were collected from a representative sample of facilities managed by the government, non-governmental organizations (NGOs), and private for-profit organizations in all 9 regions and 2 city administrations of the country.

This study provides indicators at national level for the different facility types and managing authority as well as aggregate indicators at the regional level. Data source for this study generated from the 2014 Ethiopian service provision assessment plus (SPA+) Survey.

Data collection instrument
Data were collected using a facility based inventory questionnaire to obtain information on how the facilities are prepared to provide each of the priority services.

Data Collection Approaches
Questionnaires prepared in English were translated into Amharic version by a language expert to maintain consistency. Both English and Amharic inventory questionnaire were loaded onto tablet computers, which were used during interviews (computer assisted personal interviewing – CAPI) designed using CSpro.

Sampling
The sample size for the study was determined by a combination of census and random samples. A list of 23,102 formal sector health facilities in Ethiopia was obtained from the Federal Ministry of Health. The list included: 202 hospitals, 3,292 health centres, 15,618 health posts, and 3,990 private clinics (higher clinics, Medium clinics and lower clinics) used as a sampling frame. The list includes hospitals, health centres, private for profit and for non-profit facilities, and health posts. These facilities are under various management authorities, including FMOH, NGOs, ministry of defence, Private for profit and the Federal police.

Ethiopia has a skewed population distribution at regional level. The sample allocation for the assessment took the skewed population distribution of the country into account. Out of the eleven regions, the three most populated regions represent 83% of the total population of the country (5) and thus a large proportion of facilities came from these regions. Because of their importance and their limited numbers, all hospitals were included in the survey and allowing for inclusion of newly identified hospital in the survey.

A stratified random sampling technique was employed to provide a representative data for Ethiopia, for different facility types and different management authorities, and for each of the 11 regions of the country.

Because of their importance and their limited numbers, all hospitals were included in the survey and allowing for inclusion of newly identified hospital in the survey. A representative sample of health centres and clinics were selected and included in the survey. A total of 1,327 health facilities (223 hospitals, 296 health centres, 485 private clinics and 321 health posts) were included in this study. Health posts were independently selected, analysed, and reported.

The sample size determination achieved by controlling the survey precision at regional level and by facility type at national level. The formula used for the sample size calculation is given by

\[ n = \frac{(1-p)}{e^2 p} \]

Where, \( e \) is the requested relative standard error for estimating a proportion \( p \). With the proposed sample size, for an indicator at 30% level a “good” survey precision was achieved at national level by facility type; a “good-to-acceptable” precision was achieved at regional level for the higher level health facilities, that is, facilities not including the health posts. For the health posts, only “moderate” survey precision was guaranteed at regional level since they were reported separately from the other health facilities.

<table>
<thead>
<tr>
<th>Type of Facility</th>
<th>Proposed sample size</th>
<th>Relative standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital</td>
<td>223</td>
<td>NA</td>
</tr>
<tr>
<td>Health Center</td>
<td>296</td>
<td>8.8%</td>
</tr>
<tr>
<td>Private Clinic (higher, middle, lower)</td>
<td>485</td>
<td>2.2%</td>
</tr>
<tr>
<td>Health Post</td>
<td>321</td>
<td>8.5%</td>
</tr>
</tbody>
</table>
Training and Data Collection
The questionnaires were pretested to detect any possible problems in the flow of the questionnaires, gauge the length of time required for interviews, as well as any problems in the translations. The pre-test also helped to detect any problems with the data entry programs. The main training for the survey took place from February 06, 2014 March 09, 2014. Main data collection took place from March 10, 2014, to July 25, 2014. The team leader had responsibility of checking all questionnaires before leaving the facility. Each team was given a list of facility to visit, list of facilities name, type, and location.

Data management and analysis
Data were cleaned and entered into the computer by each data collector and downloaded daily by the team supervisor. The supervisor sent the data regularly to the Ethiopian Public Health Institute (EPHI) using e-mail attachment. All data editing programs were conducted using CSPro software. Descriptive analysis was performed using CSPro tabulation. Unless otherwise indicated, the analyses considered only those items readily available and observed by the interviewers themselves during the survey.

Ethical clearance
This study was approved by the Institutional Review Board of Scientific and Ethical Review Office (SERO) of EPHI.

Informed consent was obtained from each study participant (facility in-charge, all respondents for the facility inventory questionnaires, and from interviewed providers).

Results
In total, 1,327 health facilities (223 hospitals, 298 health centres, 485 private clinics, and 321 health posts) were participated in this study. Of these, data were collected from 1,165 (88%) of the facilities. 11% of the facilities were closed or not yet operational; 1% of facilities were not interviewed for various reasons including: security reason, inaccessible for various reasons, duplication, inability to obtain consent, and facility type change (e.g. changed to a special dental clinic) (Table1).

Laboratory diagnosis strongly depends on the experience and knowledge base of the laboratory personnel; it may be less reliable in settings where laboratory personnel have less training. Tables 2 and Table 4 present information on availability of basic and advanced level diagnostic test capacity in the facility. Fifty seven percent of the facilities excluding health posts in Ethiopia have laboratory diagnostic services. HIV diagnostic test (59 %) is the most commonly provided basic laboratory services. Liver or renal function test is the least (7 %) provided laboratory service in Ethiopia. Twenty three percent of facilities excluding health posts provided haemoglobin tests, all referral hospitals (100 %), majority of general hospitals (85 % ), and quarter of the health centres (24 %) offered the test services.

Facilities managed by non-governmental organisations (40 %) offered haemoglobin test and facilities in urban areas (37%) offered the services compared to facilities in rural areas (13 %). Fifty four percent and sixty four percent of facilities in Addis Ababa Administrative Council provided haemoglobin test and blood glucose test, respectively. Afar (92 %), and Somali (79%) regions offered malaria diagnostic tests, but there is no difference in urban or rural areas (both 56 %) provided the test services. CSF/body fluid count test (45%), and stool microscopy (42%) was the most commonly provided laboratory services in almost all Ethiopian health facilities (.hospitals, higher clinics and medium clinics). Twenty percent of facilities excluding health posts provided Gram’s stain test, almost all referral hospitals and general hospitals (94 %, and majority of higher clinics (88 %) offer this test. Serum electrolyte test (10 % ), full blood count with differential (10 % ), blood typing and cross matching (3%), CD4 count (3%), syphilis serology (3%), TB culture (1%), and TB rapid tests (<1%) were the least available advanced diagnostic services in Ethiopian facilities excluding health posts.

In all regions, only less than 10 % of the existing facilities offered CD4 count test. One in four facilities in Addis Ababa, Harari, and Dire Dawa offered serum electrolyte, and full blood count tests.
Table 1: Result of facility contact, by background characteristics
Percent distribution of sampled facilities according to result of visit of the survey team to the facility, by background characteristics, Ethiopia 2014

<table>
<thead>
<tr>
<th>Background characteristics</th>
<th>Completed</th>
<th>Respondent not available</th>
<th>Refused</th>
<th>Closed/not yet operational</th>
<th>Other</th>
<th>Total percent</th>
<th>Number of facilities surveyed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facility type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Referral Hospital</td>
<td>97</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>100</td>
<td>33</td>
</tr>
<tr>
<td>General Hospital</td>
<td>97</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>134</td>
</tr>
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Note: some of the rows may not add up to 100 percent due to rounding.
## Table 2: Laboratory diagnostic capacity
Among all facilities, excluding health posts, the percentages with capacity to conduct basic and advanced laboratory diagnostic tests in the facility, by facility type, managing authority and urban/rural, Ethiopia 2014

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Note: The basic test indicators presented in this table comprise the diagnostic capacity domain for assessing general service readiness within the health facility assessment methodology proposed by WHO and USAID (WHO 2012).
Note: DBS = dried blood spot; CSF = cerebrospinal fluid; CT = computed tomography
Table 4: Among all facilities, excluding health posts, the percentages with capacity to conduct basic and advanced laboratory diagnostic tests in the facility by region, Ethiopia 2014

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<th>Gumuz</th>
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</table>

Note: The basic test indicators presented in this table comprise the diagnostic capacity domain for assessing general service readiness within the health facility assessment methodology proposed by WHO and USAID (WHO 2012).

Note: DBS = dried blood spot; CSF = cerebrospinal fluid; CT = computed tomography
Laboratory diagnostic capacities in health posts were also assessed. Fifty three and fourteen percent of the health posts provided malaria and HIV diagnostic test, respectively in Ethiopia (Table 3 and 5).

<table>
<thead>
<tr>
<th>Table 3: Laboratory diagnostic capacity</th>
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<tbody>
<tr>
<td>Among health posts, the percentages with capacity to conduct basic and advanced laboratory diagnostic tests in the facility by region, Ethiopia 2014</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Laboratory tests</td>
</tr>
<tr>
<td>Basic tests</td>
</tr>
<tr>
<td>Malaria diagnostic test</td>
</tr>
<tr>
<td>HIV diagnostic test</td>
</tr>
<tr>
<td>Number of facilities</td>
</tr>
</tbody>
</table>

Note: The basic test indicators presented in this table comprise the diagnostic capacity domain for assessing general service readiness within the health facility assessment methodology proposed by WHO and USAID (WHO 2012).

<table>
<thead>
<tr>
<th>Table 5: Laboratory diagnostic capacity</th>
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<tbody>
<tr>
<td>Among health posts, the percentages with capacity to conduct basic and advanced laboratory diagnostic tests in the facility, by facility type, managing authority and urban/rural, Ethiopia 2014</td>
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<tr>
<td>Laboratory tests</td>
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<tr>
<td>Facility type</td>
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<td></td>
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<tr>
<td>Basic tests</td>
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<tr>
<td>Malaria diagnostic test</td>
</tr>
<tr>
<td>HIV diagnostic test</td>
</tr>
<tr>
<td>Number of facilities</td>
</tr>
</tbody>
</table>

Note: The basic test indicators presented in this table comprise the diagnostic capacity domain for assessing general service readiness within the health facility assessment methodology proposed by WHO and USAID (WHO 2012).
Discussion

The capacity of a health facility to conduct laboratory diagnostic tests enhances greatly the level of service provision. Health facilities do not necessarily require the availability of a specific or designated laboratory building, but the mere presence of tests within the facility including the availability of reagents and equipment needed for each test depending on the level of the facility type. Laboratory services include the collection of specimens, laboratory tests, and rapid diagnostic tests. In this assessment, facilities may consider themselves as NOT having a laboratory if they do not have a “building” or separate site/location set aside for such services.

We are interested in the capacity of the facility to perform tests, not necessarily the building. However, if the rapid test is the only test conducted in a facility, that facility is not considered to have a laboratory, even if they have a separate building for it. If on the other hand, they do more than the rapid test, the laboratory section was completed for that facility, even if the facility does not consider the location to be a laboratory.

Provision of diagnostic services, comprising laboratory tests and diagnostic imaging, is essential for clinical decision making and for enhancing delivery of quality health care. In fact, case management for such conditions as malaria and TB depend entirely on laboratory and/or imaging results. The Ethiopia SPA+ 2013 assessed diagnostic capacity as a component of the methodology for assessing general service readiness proposed by the WHO and USAID (6).

This is the first published report on diagnostic capacity in Ethiopia and difficult to compare and discuss with other similar studies conducted in country. But, other similar studies have been conducted in other African countries. There is no methodological difference in the study conducted in those African countries but there is a time period variation. Therefore, one reason that would be observed in this comparison is due to the time period that countries conducted the survey.

The delivery of health laboratory services relies on the availability of qualified professionals, appropriate infrastructure, basic medical equipment and supplies at facility level. The results presented in this study suggest problems with availability of laboratory services, particularly in the private sector, are very low. However, as compared to other countries, the findings indicated that those facilities offering diagnostic services in Ethiopia is higher than reports from surveys of other African countries, which is 11% in Senegal (7), 7% in Sierra Leon (8), and 21% in Malawi (9), but lower than a report in Uganda (32%) (10). On the other hand, Ethiopia malaria diagnostic test (56%) is lower than from other African countries, Senegal (83%), Malawi (85%), Tanzania (73%) (11) and Uganda (89%).

Conclusions

The capacity of health facilities to conduct laboratory diagnostic tests, even the basic tests, is very low. Except for the capacity in conducting malaria and HIV diagnostic tests, for which fewer than 6 of ten facilities excluding health posts could conduct the tests, only less than half of all facilities are able to conduct the remaining basic tests. Over half of health posts are able to conduct malaria diagnostic test while only about one of ten health posts could perform HIV diagnostic test. The assessment indicated gaps in most of the laboratory services which could considerably impact quality and accessibility of laboratory diagnosis. The results of this assessment suggest the need to improve the capacity of diagnostic laboratories to fulfill basic standards and to offer quality services across health facilities in the country.

Competing interests

Authors declare there is no competing interest.

Authors’ contributions

Theodros Getachew: conception and design of study, acquisition of data, analysis, interpretation of data, and manuscript writing. Abebe Bekele: conception and design of study, coordination and manuscript writing. Atkure Defar: acquisition of data and manuscript writing. Mekonnen Tadesse: acquisition of data and manuscript writing. Habtamu Teklie: acquisition of data and manuscript writing. Kassahun Amenu: manuscript writing. Terefe Gelibo: analysis, interpretation of data and manuscript writing. Yibeltal Assefa and Amha Kebebe: overall coordination.
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Authors' Details

1'Ethiopian Public Health Institute, Addis Ababa, Ethiopia

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11. Tanzania minister of health and social welfare; Tanzania service availability and readiness assessment (SARA), 2012.
Brief Communication

Ciprofloxacin resistance among Neisseria gonorrhoea isolates obtained from genital samples referred to Ethiopian Public Health Institute in Addis Ababa, Ethiopia

Surafel Fentaw†*, Tamrat Tadesse†, Tekiil Biza†, Rajiha Abubeker†, Negga Asamene†, Meseret Assefa†, Amete Mihret† and Degefu Beyene†

Abstract
Background: Gonococcus is a major public health challenge, due to the high frequency of infections accompanied by inadequate treatment options. This study focuses on determining prevalence and antimicrobial resistance of gonococcal isolates referred to the Ethiopian Public Health Institute.
Methods: Seven hundred thirty five gonococcal isolates were obtained from patients referred to Ethiopian Public Health Institute for culture and sensitivity test between 2008 and 2014. All suspected gonococcal isolates identification and antimicrobial resistance tests were confirmed using standard microbiology techniques.
Results: Five hundred eight gonococcal isolates 69% were resistant for ciprofloxacin, which is the current antibiotic used for syndromic management of urethral discharge syndrome. The resistance pattern of another candidate antibiotics spectinimycin 3.2 %, cefoxitin 0% and Ceftriaxone 4% were for gonococcal isolates.
Conclusion: Since ciprofloxacin resistance is greater than 5% no more applicable for the national syndromic management.
Key words: Resistance, Neisseria gonorrhoea (gonococcus), Ciprofloxacin (quinolone).

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†Ethiopian Public Health Institute

Background
Sexually transmitted infections (STIs) are an important public health problem in both developed and developing countries. Untreated STIs have serious adverse health consequences including adverse pregnancy outcomes, reproductive morbidity and mortality, and enhanced HIV transmission (1). STI diagnosis and treatment are often based on laboratory testing in combination with clinical presentation. However, in resource-constrained settings laboratory testing of STIs is either unavailable or unaffordable. For these reasons, the World Health Organization (WHO) advocates the use of locally validated syndromic management approaches for diagnosis and treatment of STIs (2).
Infection caused by N. gonorrhoeae is one of the most widely disseminated STIs worldwide (3). The high rate of asymptomatic infections, under diagnosis and underreporting explains the difficulty in assessing the true incidence. Appropriate diagnosis and treatment is important to reduce drug resistance and limit its transmission (4).
Over the past decade, gonococcal resistance to the fluoroquinolones has been increasingly recognized in Africa. This represents an important reason that the current Ethiopian algorithm for the management of urethral
discharge might prove to be sub-optimal. Algorithm failure could lead to mistrust of the national syndromic treatment guidelines among patients and health providers (5). There were few studies conducted in Ethiopia. However, they were not comprehensive as they did not include the recommended drugs. In contrast, this study aimed to assess the real situations of the resistance against the recommended drugs and the isolates identified with the standard laboratory methods (6). This study attempted to determine the resistance pattern of gonococcal isolates against fluoroquinolones.

Methods
Case records of 2342 patients with suspected N. gonorrhoea who were referred to Ethiopian Public Health Institute (EPHI) from 2008 to 2014 were analyzed in this retrospective study to determine the prevalence and resistance pattern of gonococcal isolates against fluoroquinolones. Laboratory diagnosis had been done based on the standard operational procedure of EPHI laboratory (7).

Resistance: N. gonorrhoea isolates were defined as those that are not sensitive to the antibiotic tested for susceptibility, i.e., those isolates exhibiting resistance.

Intermediate: N. gonorrhoea isolates were defined as those that are not sensitive to the antibiotic tested for susceptibility with a given concentration, i.e., those isolates which are between resistance and susceptible.

Ethical Clearance
The study was approved by Scientific and Ethical Review Office (SERO) of Ethiopian Public Health Institute.

Results
Between 2008 and 2014 a total of 2342 urethral and vaginal discharge specimens were received and analyzed. Of these, 1653 (70.6%) were male and 689 (29.4%) were female. The mean age participants were 24 years with standard deviation of 6.75. The record showed that 735 (31.38) gonococcal isolates, 22 C. albicans and 12 S. agalactae were identified. Out of the total gonococcal isolates, 691 (94%) were from urethral discharge of male patients and 44 (6%) were from cervical discharge of female patients. The gonococcal culture positivity rate among male patients was 691/1653 (42%) and 44/689 (6.3%) among females. Besides gonococcus, female patients were culture positive for C. albicans and S. agalactae (Table 1) but no men were positive for such isolates.

Table 1 Culture positivity rate of Urogenital tract infection at EPHI 2008-2014

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=1653)</td>
<td>(n=689)</td>
</tr>
<tr>
<td>N. gonorrhoea</td>
<td>691</td>
<td>44</td>
</tr>
<tr>
<td>C. albicans</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>S. agalactae</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>962</td>
<td>611</td>
</tr>
</tbody>
</table>

The distribution of antimicrobial susceptibility pattern of 735 gonococcal isolates tested against first line therapeutic agent and alternative second line antimicrobial agents are shown in Table 2.

Five hundred seven gonococcal isolates 507/737 (69%) were resistant for ciprofloxacin / florquinolone which is the first line of therapeutic agent for urethral discharge syndrome. Other potential candidate antibiotics (third generation cephalosporin and amoxicyclitol) have resistance pattern of 3.8% and 4% respectively. The proportion of multiple antibiotics resistance (resistance for two or three tested antibiotics) isolates accounts about 2% (15/735).

Table 2 Antimicrobial susceptibility result of Gonococcal isolates at EPHI 2008-2014

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>R (%)</th>
<th>I (%)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cipro</td>
<td>63</td>
<td>6</td>
<td>31</td>
</tr>
<tr>
<td>Spect</td>
<td>0</td>
<td>3.2</td>
<td>96.8</td>
</tr>
<tr>
<td>Ceftr*</td>
<td>4</td>
<td>-</td>
<td>96</td>
</tr>
<tr>
<td>Cefox</td>
<td>0</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>Cipro + Ceftr</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cipro + Spect</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftr + Spect</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*No intermediate interpretation for Ceftriaxone
Cipro-Ciprofloxacin; Spect-Spectinomycin; Ceft-Ceftriaxone; Cefox-Cefoxitin; R-Resistant; I-Intermediate; S-Susceptible

Discussion
The general culture positive rate in this study was around 33% (769/2342) for all isolates 31% (735/2342) for N. gonorrhea only. The gonococcal culture detection rate was low compared with other studies 50% in Kenya 35% in Uganda. The low gonococcal culture detection rate might be due to other confounding factors since patients are referred due to treatment failure (prior antibiotic treatment may inhibit growth of culture, or other sexually transmitted infections besides N. gonorrhea) (8,9).

Old antibiotics: Sulfonamides, Penicillin and Tetracycline were not tested against N. gonorrhea isolates. But penicillinase producing gonococcal isolates were detected while performing biochemical identification using API-NH®. Out of 735 gonococcal isolates 332 isolates were penicillinase (beta-lactamase) positive by API-NH® test (10).
Nationally fluoroquinolone therapy was considered first line therapy for treatment of gonorrhea for a number of years. Even though, the World Health Organization (WHO) advocates the use of locally validated syndromic management approaches for diagnosis and treatment of STIs but prevalence of resistance should be less than the threshold of 5%. It was long time since the previous study. Nationally the syndromic management was started since 2006 after doing validation study at 8 Health centers in Addis Ababa and one health center in Hawassa. The recommended first line treatment for urethral discharge syndrome was fluoroquinolone single dose (oral) ciprofloxacin. (3, 11)
The ciprofloxacin resistance rate was around 69% (63% resistant and 6 % intermediate) this result agrees with other studies in the East African, Southern African Region and South East Asia region. The Kenyan study showed that quinolone resistant in the country ranges between 11% to 81% and in Uganda the quinolone resistance among female sex workers was 83%. This indicates that the emergence of floroquinolone resistance is spreading in different regions in alarming rate (6, 8, 9).

The next probable candidate of antibiotics ceftriaxone (third generation cephalosporin) or Spectinomide (aminocyclitol) have the susceptibility of 96% and 96.2%. Since Ceftriaxone or Spectinomide have resistance of less than 5%, these candidate antibiotics could be used as first line of therapeutic agents in syndromic management (10, 11). Currently most countries have introduced oral or injectable cephalosporin in combination with single dose (oral) azithromycine. In some places cephalosporin resistance has been reported. Combined therapy injectable ceftriaxone 250 may slow the emergence of resistant N. gonorrhea (12).

Conclusion
Since the resistance rate of floroquinolone/ciprofloxacin resistance was 69%, (Table 2) this candidate antibiotic is no more recommended for syndromic management. Another alternative algorithm should be initiated by policy makers and responsible stakeholders.

Recommendation
The current guideline should be revised based on the finding. Floroquinolone should be replaced with other alternative potential cost effective antibiotics Periodic surveillance of gonococcal distribution and antimicrobial resistance is important.

Limitation of the study
Samples may not be nationally representative. Other etiologic agents besides N. gonorrhea were not addressed. More demographic data were missed.

Competing interests
The authors declare that they have no competing interests.

Authors’ contribution
SF, Principal investigator of the study, study design, data collection, laboratory work, write up and data analysis; TT, TB, RA, NA, MA, AM, and DB data collection and laboratory work; All
authors commented and approved the final manuscript.

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10. API-NH manufacturer leaflet
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