STUDY ON ANTIMICROBIAL RESISTANCE PATTERN IN A TERTIARY CARE HOSPITAL – AN OBSERVATIONAL RETROSPECTIVE STUDY

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ABSTRACT

Objective: The aim of the study was to monitor the magnitude and trends in antimicrobial resistance through surveillance programs and bacteriological diagnostic methods.

Methods: The study research has performed a retrospective observational study for period of 6 months (Dec 2019–June 2020) to determine the antimicrobial susceptibility pattern on cultures of various specimens at Vivekanandha Medical Care Hospital. The resultant data were computed in Microsoft Excel. Analyses were expressed as the total number, frequencies, and percentage.

Results: A total of 110 culture and sensitivity reports were collected from microbiology departments. Among the culture report, 84% accounted for gram-negative and 16% for Gram-positive organisms. Out of eight specimens, 15 different bacteria were identified and the most common bacteria were *Klebsiella pneumonia* (25%), *E. coli* (14%), *Staphylococcus aureus* (13%), and *Pseudomonas aeruginosa* (12%).

Conclusion: The interferences from the present study can help in bridging the gap in future educations, training, and adequate decision-making.

Keywords: Antibiotic, Culture, Sensitivity reports, Microbial resistance.

INTRODUCTION

Antibiotics are essential medicines that combat bacterial infections with the aid of disrupting the method essential for bacterial cell growth and proliferation. They represent one of mankind's crucial medical inventions saving millions of lives throughout the years. The first broad-spectrum antibiotic introduced into the world, Penicillin was discovered by Alexander Fleming in 1928. Penicillin has saved and is still saving millions of lives worldwide playing a major role in treating bacterial diseases and in the creation of today's pharmaceutical industry [1,2]. On receiving the Nobel Prize on 1945, December 11, Alexander Fleming has warned regarding the abuses of penicillin one may impose on themselves and on others when they are not used appropriately, and with this thoughtless behavior comes the consequence. Adding to the statement, "The time may come when penicillin can be bought by anyone in the shops."

The genetic ability of resistant microbe to exhibit inhibitory action on antibiotics by resistance genes gives rise to the phenomena called anti-microbial resistance (AMR) [3]. AMR is a severe health issue concern with the extreme impact on the global health sector and economy. They are the subsequent results of evolution through natural selection or we can say antibiotic action. A large part of resistant pathogenic organisms in a micro-biosphere is pushed due to human activities, consisting of indiscriminate use and misuse of antibiotics in humans, veterinary medication, and agriculture [4,5]. At the same time, when high concentrations of antibiotics used therapeutically can create an environment for resistant mutants challenging minor and primary infectious.

Many patients are given a non-specific broad-spectrum medicament for an actual or suspected infection which is often not necessary to the given condition. Subsequently, determining the resistance patterns inside the geographical area will assist in choosing and use of the effectiveness of antibiotics.

METHODS

The current research performed an observational retrospective study for 6 months (December 2019–June 2020) to determine the antimicrobial susceptibility pattern on cultures of various specimens collected over 2 years. Data on the identified micro-organisms and their antimicrobial susceptibility pattern were obtained from the Clinical Microbiology Laboratory of Vivekanandha Medical Care teaching Hospital (VMCH). The data regarding culture and sensitivity (C/S) were isolated from the different specimens such as pus, blood, wound swab, conjunctival, tracheal, pleural fluid, sputum, and urine and were analyzed including out-patients (OP) and in-patients (IP). To eliminate bias in the collection of research data, the C/S reports were filtered to retain only the first isolate of the patient. The report was computed in Microsoft Excel and was expressed as proportions and percentages. The research protocol was approved by the Institutional Ethical Committee, with the approval number SVCP/IEC/JAN/2020/02.

RESULTS

The total 110 culture and sensitivity reports had shown (46.2%) significant growth of pathogenic organisms exhibiting resistance to either single or multiple antibiotics.

Distribution of bacteria as per Gram-stain
Among the culture reports collected, 84% were Gram-negative organisms and 16% accounted for Gram-positive organisms as depicted in Fig. 1.

Distribution of identified bacterial pathogens
The number of isolates obtained was higher from urine culture (n=45), followed by Pus (n=23), sputum (n=17), tracheal (n=9), and blood (n=8). In contrast, the least number of isolates was found in wound swab (n=4), ear swab (n=3), and conjunctival (n=1) as represented in Table 1.

Organisms isolated from various samples were *Enterococcus*, *Enterobacter baumannii*, *Streptococcus pneumonia*, *Proteus*, *Acetobacter*, *Staphylococcus hominis*, *Enterobacter kobei*, *Klebsiella oxytoca*, *Corynebacterium striatum*, *Pseudomonas aeruginosa*, and *Escherichia coli.*

Out of eight specimens, fifteen different bacteria were identified, and the most frequently isolated microorganisms were *Klebsiella pneumonia* (n=27)*, E. coli* (n=15), *Staphylococcus aureus* (n=14), and *Pseudomonas aeruginosa* (n=13) as depicted in Table 1.

In percentile, *Klebsiella pneumonia* was the most common organism isolated with 47%, followed by *Escherichia coli* (22%), P*seudomonas aeruginosa* (13%), A*cinetobacter baumannii* (5%), and P*roteus mirabilis* (4%) in the Gram-negative organisms. Among Gram-positive organisms, S*taphylococcus aureus* (37%), C*andida tropicalis* (25%), and E*nterococcus faecalis* (15%) were frequently isolated.

As shown in Fig. 2, *Klebsiella pneumoniae* (n=17) and *Escherichia coli* (n=15) were the most frequent bacteria isolated from the urine culture,
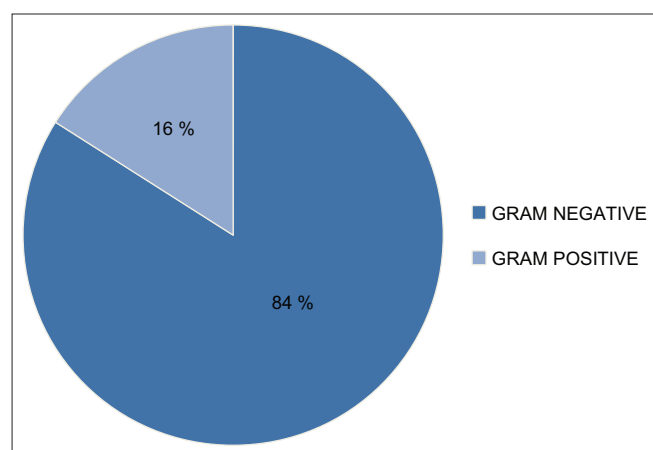
Staphylococcus aureus (n=10) and *Streptococcus pneumoniae* (n=6) were found from pus and *Pseudomonas aeruginosa* (n=5) from sputum culture. *Staphylococcus aureus* (n=3) was the most seen organism in the blood samples.

Streptococcus pneumoniae (n=1) was the only organism that was identified in the conjunctival culture test, likewise *Corynebacterium striatum* (n=2) in-ear swab. *Pseudomonas aeruginosa* (n=2) was majorly seen in wound swab culture. *Klebsiella pneumoniae* (n=2), *Candida tropicalis* (n=2), and *Enterococcus faecalis* (n=2) from tracheal culture test.

Resistant frequency of bacterial isolates

Among the tested antimicrobials toward Gram-negative and Gram-positive organisms, the study showed more resistance to ampicillin (n=36) accompanied with co-trimoxazole (n=28), ceftriaxone (n=26), and cefixime (n=24). The least resistance was shown by tigecycline (n=6), clindamycin (n=4), and cefoperazone (n=2), as represented in Tables 2 and 3.

From seven different pharmacological categories, 37 antimicrobial agents showed resistance towards various organisms. Third-generation cephalosporins (28%) exhibited superior resistance, followed by penicillin (27%). Other agents were fluoroquinolones (14%), oral and parenteral aminoglycosides (5%), tetracycline (5%), and macrolides antibiotics (8%) along with nitrofurantoin, colistin, rifampin, and co-trimoxazole.

Table 3 depicts the resistance frequency of bacteria toward anti-microbial whereas Table 4 represents the resistance percentile of micro-organisms exhibit towards antibiotics. *Escherichia coli* have shown 53% resistance to ciprofloxacin and 47% resistance to ceftriaxone, co-trimoxazole. *Klebsiella pneumoniae* showed higher resistance to Ampicillin by 67%. *Pseudomonas aeruginosa has shown* 33% resistance to co-trimoxazole, ceftriaxone, cefixime, and levofloxacin*.* Whereas, *Staphylococcus aureus* was with the highest resistance to ampicillin at 43%.

DISCUSSION

The research aimed to study the identified organisms and to understand their antibiotic susceptibility pattern as per the culture and sensitivity
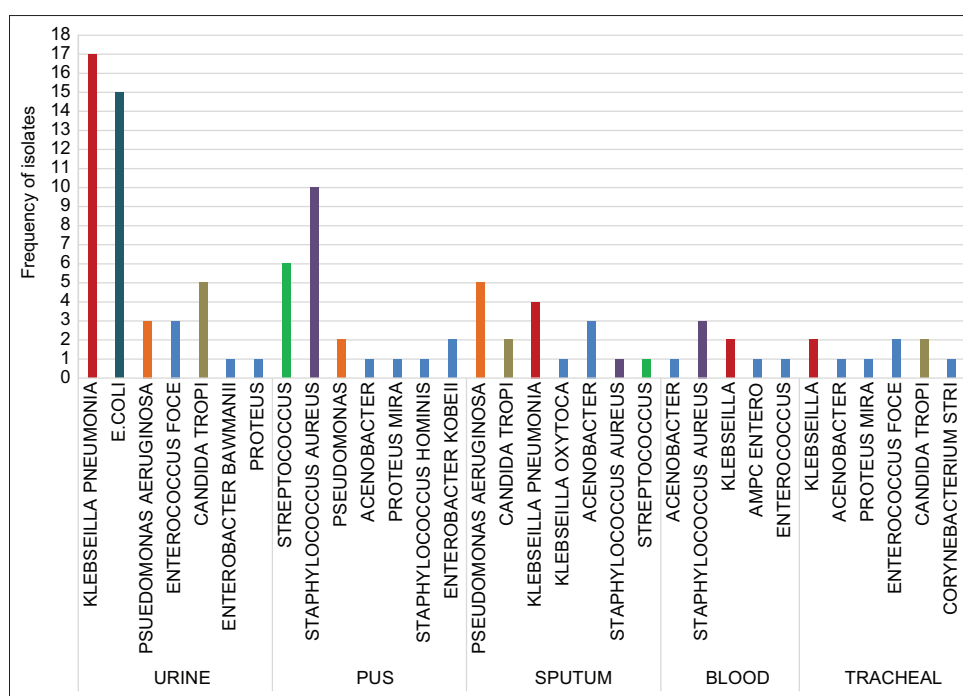

Fig. 1: Distribution of bacteria as per gram stain`

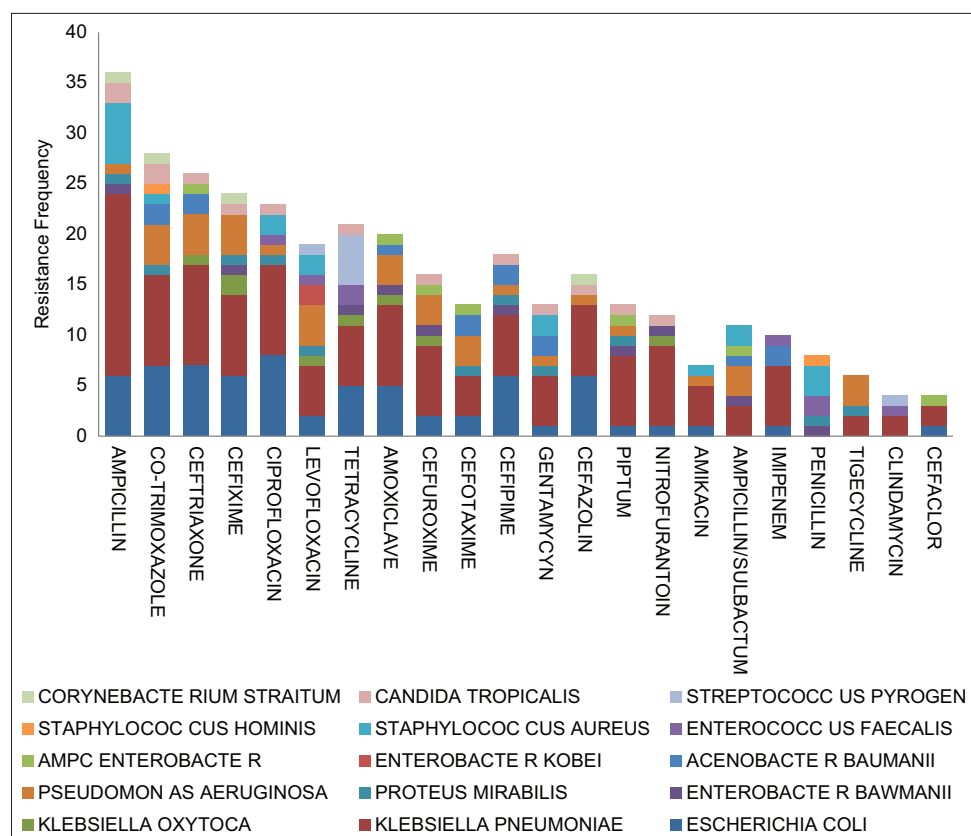**Fig. 2: Organisms in the collected specimens**

Fig. 3: antibiotic resistance against micro-organism

Table 1: Distribution of identified bacterial pathogens

Type of isolates	Type of specimen							
	Urine	Pus	Sputum	Blood	Conjunctival	Ear	Wound	Tracheal
K. pneumonia	17	-	4	2	-	-	2	2
E. coli	15	-	-	-	-	-	-	-
P. aeruginosa	3	2	5	-	-	1	2	-
Enterococcus	3	-	-	1	-	-	-	2
Candida	5	-	2	-	-	-	-	2
E. bawmanii	1	-	-	-	-	-	-	-
Proteus	1	1	-	-	-	-	-	1
Streptococcus pneumonia	-	6	1	-	1	-	-	-
S. aureus	-	10	1	3	-	-	-	-
Acetobacter	-	1	3	1	-	-	-	1
S. hominis	-	1	-	-	-	-	-	-
Enterobacter kobei	-	2	-	-	-	-	-	-
Klebsiella oxtoca	-	-	1	-	-	-	-	-
Ampc enterobacter	-	-	-	1	-	-	-	-
Cornebacteriumstriatus	-	-	-	-	-	2	-	1

(C/S) reports collected from the clinical microbiological laboratory of VMCH. As per the results obtained, Gram-negative organisms (GNO) had the highest predominance accounting for 84% of total C/S collection, and Gram-positive organisms (GPO) with 16%. The predominance of GNO in hospital settings is similarly seen in other studies of Mehdinejad *et al.*, Ali Shamshad *et al.,* and Melkamu *et al.* [6-9]. In contrast, other studies had reported GPO as the predominant pathogen in studies conducted by Kalpesh *et al.*, Gupta, and Karlowsky *et al.* [10-12]. According to studies conducted by Tiwari *et al.* and Mathai *et al.* the highest rates of Gram-negative bacillary resistance was found in India and it is well-known that GNO is more frequently encountered in different clinical specimens causing nosocomial infections (sepsis, pneumonia, and meningitis) [13,14].

The predominant Genus/Species in Gram-Negative isolates are *Klebsiella (25%), E. coli (14%),* and *Pseudomonas species (12%).* Karlowsky *et al.*, Kalpesh *et al.*, Gupta *et al.*, and Kamga *et al.* have reported a high incidence of *Klebsiella* and *E. coli.* [10-12]. Likewise, in Gram-positive organisms, *Staphylococcus aureus (13%)* has shown the highest frequency. This report was consistent with the study of Bhatia *et al.*, Tiwari *et al.*, Kalpesh *et al.*, and Karlowsky *et al.* where the isolation of the organism was reported [10,12,51,52].

As per the finding, the maximum clinical isolates obtained were from urine specimens (45) followed by pus (23), sputum (17), and tracheal (9). The result was comparable with the reports of Alnamnakani *et al.* [15].

Table 2: Resistant frequency of gram-negative bacterial isolates

Organism	Ampicillin	Co-trimoxazole	Ceftriaxone	Cefixime	Ciprofloxacin	Levofloxacin	Tetracycline	Amoxiclav
GNO								
E. coli	6	7	7	6	8	2	5	5
K. pneumoniae	18	9	10	8	9	5	6	8
Klebsiella oxytoca	0	0	1	2	0	1	1	1
Enterobacte R bawmanii	1	0	0	1	0	0	1	1
Proteus mirabilis	1	1	0	1	1	1	0	0
Pseudomonas aeruginosa	1	4	4	4	1	4	0	3
Acenobacterbaumanii	0	2	2	0	0	0	0	1
Enterobacter kobei	0	0	0	0	0	2	0	0
Ampcenterobacter	0	0	1	0	0	0	0	1

Organism	Cefuroxime	Cefotaxime	Cefipime	Gentamycyn	Cefazolin	Piptum	Nitrofurantoin	Amikacin
GNO								
E. coli	2	2	6	1	6	1	1	1
K. pneumoniae	7	4	6	5	7	7	8	4
Klebsiella oxytoca	1	0	0	0	0	0	1	0
Enterobacte R bawmanii	1	0	1	0	0	1	1	0
Proteus mirabilis	0	1	1	1	0	1	0	0
Pseudomonas aeruginosa	3	3	1	1	1	1	0	1
Acenobacterbaumanii	0	2	2	2	0	0	0	0
Enterobacter kobei	0	0	0	0	0	0	0	0
Ampcenterobacter	1	1	0	0	0	1	0	0

Organism	Ampicillin/ sulbactum	Penicillin	Tigecycline	Clindamycin	Cefaclor	Nalidixic acid	Norfloxacin
GNO							
E. coli	0	0	0	0	1	1	1
K. pneumoniae	3	0	2	2	2	2	2
Klebsiella oxytoca	0	0	0	0	0	0	0
Enterobacte R bawmanii	1	1	0	0	0	0	0
Proteus mirabilis	0	1	1	0	0	0	0
Pseudomonas aeruginosa	3	0	3	0	0	0	0
Acenobacterbaumanii	1	0	0	0	0	0	0
Enterobacter kobei	0	0	0	0	0	0	0
Ampcenterobacter	1	0	0	0	1	0	0

K. pneumoniae was found to be the major isolate from urine samples and identified etiologic agent following *E. coli* which was similar to the findings of Alnamnakani *et al.*, Bajaj *et al.*, Sheth *et al.*, Javeed *et al.*, Beyene *et al.*, Tadesse *et al.*, and Mukhtar *et al.*, other studies conducted globally [15,16-21].

Amongst the pus culture samples, Streptococcus pneumonia and *Staphylococcus aureus* were the most common bacterial agents. Similarly, studies conducted by Alnamnakani *et al.* and Tiwari *et al.* have reported *Staphylococcus aureus* as the most common organism [13,15]. *Staphylococcus aureus* was the most frequently isolated microbe in the blood samples collected during the study period. The findings from previous studies on Ventricular Associated Pneumonia (VAP), Surgical Site Infections (SSI) associated patients, and pediatric populations are in agreement with the above data, that is, *E.* coli, *K. pneumoniae,* and *Staphylococcus aureus* were the major pathogenic organisms. Beyene *et al.*, Tadesse *et al.*, Rao *et al.*, Sandeep *et al.*, Seni *et al.*, Lubega *et al.*, Abebe *et al.*, and Alnamnakani *et al.* [9,15,19,20,25-28]. These reports support the current findings that these organisms are known etiologies for both community and nosocomial associated infections.

The highest percentage of resistance in *Escherichia coli* causing infections were found to be co-trimoxazole (47%), ceftriaxone (47%), cefixime (40%), ampicillin (40%), cefotaxime (40%), and cefazolin (40%) in the sample population. The finding analysis of the current study showed

1st, 3rd, and 4th generation cephalosporin has shown less sensitive compared to the 2nd generation cephalosporin. The study findings were in line with other reports in Ethiopia and overseas.[40-43] Antibiotics that have retained their usefulness and showed the least resistance to the Gram-negative isolate; *E. coli* were gentamycin, piptum, imipenem, and nitrofurantoin at 93% and Amikacin at 83%. Comparable data were found in the Divyashanthi *et al.* study [37]. Apart from cephalosporin, ciprofloxacin has shown a higher resistance percentile of 53% followed by co-trimoxazole (47%), and nalidixic acid (27%) in our study. This pattern of resistance was shown by studies conducted by Deep *et al.*, Maksum *et al.*, Barai *et al.*, and other researches [37-42].

Klebsiella sp. showed the highest level of resistance to Ampicillin (67%). The resistance shown by *Klebsiella* was also observed in other studies conducted by Sheth *et al.*, Javeed *et al.*, and Divyashanthi *et al.* [17,18,37]. Likewise, the sensitivity pattern was seen highest at 93% to norfloxacin, nalidixic acid, cefaclor, clindamycin, and tigecycline followed by gentamycin (82%), ceftriaxone (68%), co-trimoxazole, and ciprofloxacin at 67% similarly cefixime and nitrofurantoin at 70%. The sensitivity pattern observed was much higher than the studies conducted globally in Gondar, and Ethiopia [9,44].

Findings of the current study showed *Klebsiella pneumonia* had the highest susceptibility rate for levofloxacin (82%), cefotaxime (85%), gentamycin (82%), amikacin (85%), ampicillin/sulbactam (89%),

Table 3: Resistant frequency of gram positive bacterial isolates

Organism	Ampicillin	Co-trimoxazole	Ceftriaxone	Cefixime	Ciprofloxacin	Levofloxacin	Tetracycline	Amoxiclave
GPO								
Enterococcus faecalis	0	0	0	0	1	1	2	0
Staphylococcus aureus	6	1	0	0	2	2	0	0
Staphylococcus hominis	0	1	0	0	0	0	0	0
Streptococcus pyrogen	0	0	0	0	0	1	5	0
Candida tropicalis	2	2	1	1	1	0	1	0
Corynebacte-riumStraitum	1	1	0	1	0	0	0	0

Organism	Cefuroxime	Cefotaxime	Cefepime	Gentamycin	Cefazolin	Piptum	Nitrofurantoin	Amikacin
GPO								
Enterococcus faecalis	0	0	0	0	0	0	0	0
Staphylococcus aureus	0	0	0	2	0	0	0	1
Staphylococcus hominis	0	0	0	0	0	0	0	0
Streptococcus pyrogen	0	0	0	0	0	0	0	0
Candida tropicalis	1	0	1	1	1	1	1	0
Corynebacte-riumStraitum	0	0	0	0	1	0	0	0

Organism	Ampicillin/sulbactum	Penicillin	Tigecycline	Clindamycin	Cefaclor	Nalidixic acid	Norfloxacin
GPO							
Enterococcus faecalis	0	2	0	1	0	0	0
Staphylococcus aureus	2	3	0	0	0	0	0
Staphylococcus hominis	0	1	0	0	0	0	0
Streptococcus pyrogen	0	0	0	1	0	0	0
Candida tropicalis	0	0	0	0	0	0	0
Corynebacte-riumStraitum	0	0	0	0	0	0	0

Table 4: Resistance percentile of bacteria

Antibiotics tested	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Ampicillin	40	67	8	43
Co-trimoxazole	47	33	31	7
Ceftriaxone	47	37	31	_-
Cefixime	40	30	31	_-
Ciprofloxacin	53	33	8	14
Levofloxacin	13	18	31	14
Tetracycline	33	22	-	_-
Amoxiclav	33	30	23	_-
Cefuroxime	13	26	23	_-
Cefotaxime	13	15	23	_-
Cefepime	40	22	8	_-
Gentamycyn	7	18	8	14
Cefazolin	40	26	8	_-
Piptum	7	26	8	_-
Nitrofurantoin	7	30	_-	_-
Amikacin	7	15	8	7
Ampicillin/sulbactum	-_	11	23	14
Imipenem	7	22	-	-
Cefaclor	7	7	_-	_-
Nalidixic acid	27	7	_-	_-
Norfloxacin	20	7	_-	_-

tigecycline, clindamycin, cefaclor, nalidixic acid, and norfloxacin at 93%. The above finding was relatively higher as compared to other reports done in Ethiopia and Gondar [9,44]. Whereas, moderate susceptibility was shown by cefexime (70%), tetracycline (78%), amoxyclav (70%), cefuroxime (74%), cefepime (78%), cefazolin (74%), piptum (74%), and carbapenem (74–78%). The least sensitivity was observed for ceftriaxone (63%), co-trimoxazole (67%), and ciprofloxacin (67%).

About 22% of total 14 showed moderate sensitivity towards penicillin antibiotic. In addition, 86% of isolates have shown higher susceptibility to fluoroquinolones, gentamycin, and ampicillin/sulbactam. The highest sensitivity percentile was observed for amikacin and co-trimoxazole at 93%

Among the *Pseudomonas aeruginosa* isolates in the current study, aminoglycosides (92%), and ciprofloxacin (92%) showed the higher sensitivity. The above result data were in line with Divyashanthi *et al.*, Nyambura *et al.*, Sheth *et al.*, and BasantiPathi *et al.* [17,22,37,48]. Likewise, moderate sensitivity pattern were shown by co-trimoxazole (69%), ceftriaxone (69%), levofloxacin (69%), cefuroxime (77%), cefotaxime (77%), and ampicillin/sulbactam (77%). The previous other studies conducted in Ethiopia and overseas had also conducted C/S reports on *Pseudomonas* sp. [33-36].

Antibiotics that had retained their usefulness and showed greater susceptibility pattern to these Gram-negative isolates in the study were amikacin (83%/85%/92%), gentamicin (93%/82%/92%), levofloxacin (87%/82%/69%), piptum (93%/74%/92%), cefaclor (93%/93%/92%) against *E. coli*, *Klebsiella,* and *Pseudomonas,* respectively. This was similar to the previous study reports [17,18,37,46,47].

CONCLUSION

The study was able to observe and provide an epidemiological variation in resistance and sensitivity pattern by comparing the previous reports. To decrease the rising antibiotic resistance, rational use of antibiotics is essential according to the antibiotic resistance pattern of that particular institution. For this concern, a regular epidemiological study needs to be conducted on C/S isolates to guide the clinicians in the selection of the appropriate empirical therapy and switching over to the best therapeutic choice based on the data. In addition, study data can be used as a baseline for future analysis to establish prescribing guidelines, determining new therapies, and improve the focus of education and campaigns in reducing AMR.

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