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CHANGING TRENDS OF ANTIBIOTIC SENSITIVITY PROFILE AMONG GRAM NEGATIVE BACTERIA ISOLATED FROM URINE SAMPLES IN CRITICAL CARE PATIENTS OF A TERTIARY CARE HOSPITAL

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ABSTRACT
Urinary tract infections (UTIs) are among the most frequent infections in humans, particularly in intensive care units (ICUs) due to impaired immunity and invasive procedures. The changing resistance patterns of bacteria pose a major public health concern, often leading to therapeutic failure. This study aimed to determine the prevalence of UTIs among ICU patients, identify the bacterial profile, assess antimicrobial sensitivity, and evaluate ESBL, AmpC, and MBL production among multidrug-resistant (MDR) Gram-negative bacilli.
A total of 1,908 clinical samples were processed over four years, 634 (33.2%) samples showed positive growth, of which only 527(89%) yielded Gram negative bacteria , out of which 427 MDR Gram-negative were selected for further study. Identification and antimicrobial susceptibility testing were performed, followed by screening for β-lactamase production. Among the isolates, *Escherichia coli* was most common (60%), followed by *Klebsiella pneumoniae* (19%) and *Pseudomonas aeruginosa* (12%). *E.coli* showed maximum sensitivity to Ceftazidime (24%) among third-generation Cephalosporins, Norfloxacin (24%) among fluoroquinolones, Amikacin (63%) among aminoglycosides, and meropenem (68%) among Carbapenems. Sensitivity to the BL-BLI combination Piperacillin/Tazobactam was 56%. *Klebsiella pneumoniae* demonstrated 20% sensitivity to Ceftazidime, 23% to Ciprofloxacin, and 65% to Gentamicin.
β-lactamase production was detected in 340 strains. Of these, 175 (40.9%) were ESBL producers, 106 (24.8%) AmpC producers, and 59 (13.8%) metallo-β-lactamase (MBL) producers. Co-production of ESBL, AmpC, and MBL was observed in 102 (23.9%) strains, predominantly in *E. coli*. The high prevalence of β-lactamase producing isolates in ICU patients highlights the urgent need for continuous surveillance, strict antibiotic stewardship, and robust infection control measures to mitigate the growing burden of antimicrobial resistance.

KEYWORDS: Multidrug- resistant, Gram-negative bacilli, ICU, UTI, Antimicrobial resistance, ESBL, Carbapenemase , AmpC

INTRODUCTION

The incidence of nosocomial infections in the intensive care units (ICU) is showing a rising trend, mainly because of the severe clinical conditions which are associated with the impaired immunity, increasing the use of invasive diagnostic procedures, lapses in the sterilization and the disinfection techniques and the indiscriminate use of antibiotics. Hospital-acquired urinary tract infections (UTIs) are the most frequent nosocomial infections and are responsible for 20—30% of nosocomial infections in medical or surgical intensive care units (ICUs) (Richard et al., 2000). The resistant bacteria are emerging worldwide as a threat to the admitted patients. Among Enterobacteriacae most frequent ones are *Klebsiella pneumoniae* followed by *Escherichia coli*, *Enterobacter* species and *Proteus*. Among non fermenters *Pseudomonas aeruginosa* and *Acinetobacter baumanii* are the common isolates in Microbiology laboratory. Once these organisms are established as the source of infection, they are aggressively treated with antimicrobial drugs. Improper usage of antimicrobials complicates the problem of health care associated infections by encouraging multi drug resistance in nosocomial pathogens and treatment options are fast running out, particularly against Gram negative nosocomial pathogens (Russo et al., 2008). β - lactamases production by several Gram negative organisms is the most important single mechanism of resistance to Penicillin and Cephalosporins. Cephalosporins once believed to be relatively immune to attack by β- lactamases have shown resistance by way of production of extended spectrum Beta lactamases (Ayyagari et al., 2001). As the newer antibiotics were developed to overcome the problems of resistance against available antibiotics bacteria developed mechanism to resist the newer antimicrobials by way of alteration of target sites for binding of β-lactams and production of new enzymes against antibiotics. To overcome the problem of resistance Carbapenems were introduced but no sooner they were brought into use bacterial population started producing Carbapenemase which hydrolyze Carbapenem. The high resistance for antibiotics and the rapid dissemination of Metallo beta lactamase

producing isolates in hospitals require an effective Phenotypic method for identifying them as there are no prescribed CLSI guidelines available for detecting them (Tamil et al., 2006). Molecular class B enzymes have zinc in their active site so the catalytic activity of this enzyme can be enhanced for detection by incorporating it for the tests, unlike serine carbapenem resistance they are not inhibited by clavulanic acid, sulbactam and tazobactam (Walsh et al.,2005).

Hence there is the need to design an antimicrobial regime active against all Gram negative bacteria, as these organisms are highly efficient at acquiring genes that code for mechanism of antibiotic resistance especially in the presence of antibiotic pressure. The study of changing trends of antibiotic sensitivity profile among Gram negative bacteria , its` Phenotypic characterization will help in surveillance of resistance to β- lactam antibiotic which in turn will help in hospital infection control and prevention and dissemination of resistance strain. The study will help to organize intervention measures in infection control in the ICU's.

MATERIAL AND METHODS

This cross-sectional study, conducted in the Department of Microbiology, on critical care patients of Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Sri Amritsar and Acharya Shri Chander College of Medical Sciences and Hospital, Sidhra, Jammu. Of the total 1908 clinical samples, obtained over a period, of four years (from January 2019 to December 2022), 634 (33.2%) samples shows positive growth, of which only 527(89%) samples yielded Gram negative bacteria. In this study we have selected only multi drug resistance Gram negative bacterial strains those were resistance to two or more unrelated classes of antibiotics n= 427 (81.02%) and excluded the strains of GPC and some GNB showing higher sensitive pattern from this. All the isolates were identified by the standard microbiological tests. The antimicrobial susceptibility testing of the isolates was determined by the Kirby Bauer disc diffusion method according to the CLSI guidelines (CLSI 2022). AST was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar as per Clinical and Laboratory Standards Institute (CLSI) (2022).

Antibiotics used for Gram negative bacilli were Ampicillin(10µg), Amoxy/Clav (20/10µg), Piperacillin (MIC), Piperacillin-Tazobactum(100/10µg),Ticarcillin(75µg),Gentamicin(10µg),Amikacin(30µg),Tobramicin(10µg), Fosfomycin(20µg),Norfloxacin(10µg),Nitrofurantoin(300µg),Ciprofloxacin(5µg),Ofloxacin(5µg),TrimethoprimSulphamethoxazole(1.25/2.75µg),Tigecycline(15mcg),Cefazolin(30µg),Ceftazidime(30µg),Cefotaxime(30µg),Ceftriaxone(30g),Cefepime(30µ),Cefpodoxime(10µg),Cefuroxime(30µg),Cefoxitin(30µg),CeftriaxoneEDTASulbactam(30/100/15µg),Ceftazidime/Clavunate,Ceftazidime/Avibactum(30/20ug),Cefoperazone -Sulbactam, Imipenem (10 µg),Meropenem(10 g), Colistin Or Poly B (MIC). The reference strains, ESBL positive Klebsiella pneumonia ATCC 700603 and ESBL negative Escherichia coli ATCC 25922 were included in the study. Gram negative bacteria with resistance or decreased susceptibility to third generation Cephalosporins were tested for ESBL production by following methods:

Detection of the ESBLs – Each strain was screened for the ESBL production against Cefotaxime, Ceftazidime and Cefpodoxime. The strains which were resistant to these third generation Cephalosporins were confirmed by phenotypic tests

i) Double disc synergy test (DDST)- Discs of Aztreonam, Cefotaxime, Ceftazidime, Cefpodoxime were placed 15 mm from Amoxy-Clavulanic acid disc. Enhancement of zone of inhibition towards Amoxy-clavulanic acid disc was taken as ESBL positive (Ananthakrishnan et al., 2000).

ii) Disc potentiation test - Lawn culture of standard suspension of isolates was inoculated on Mueller Hinton agar plate. Discs of Ceftazidime (30 µg), Ceftazidime – Clavulanic acid (30/ 10 µg) and Cefotaxime (30 µg), Cefotaxime - Clavulanic acid (30/ 10 µg) were placed on culture plate. 5mm enhancement of the inhibition zone of antibiotic/ clavulanic acid combination vs antibiotic tested alone was taken as ESBL producers (CLSI 2022).

Detection of the AmpC β-lactamases – All the strains were screened for the AmpC β-lactamase production by the disc antagonism test. The isolates which showed a reduced susceptibility to Cefoxitin were tested for confirmation by the modified three dimensional test.

i) Modified three dimensional test - Lawn culture of *Escherichia coli* ATCC 25922 was prepared on Mueller Hinton agar plate. Cefoxitin disc (30 µg) was placed on the plate. linear slits (3 cm) were cut using sterile surgical blade 3 mm away from Cefoxitin disc. 30 µl of bacterial enzyme extract was loaded into each slit. After incubation at 37^0C, enhanced growth of surface organism at point where the slit intersected the zone of inhibition of Cefoxitin disc, causing clear distortion, was interpreted as positive AmpC enzyme. (Singhal et al. 2005).

Detection of the Metallo- β- lactamases (MBLs) – Isolates resistant to Carbapenems were tested for MBL production . Confirmatory test was done by one of the methods:

i) Disc potentiation test

The organism was inoculated on Mueller Hinton agar plate. Two (10 µg) Imipenem discs were placed on the plate and 10 µl of EDTA solution was added to one of them. Plates were incubated at 35° C for 16-18 hours. If the increase in the inhibition zone with the Imipenem and EDTA disc was more than 7 mm than the Imipenem disc alone, it was considered as MBL positive (Behera et al 2005).

ii) Modified Hodge test for Carbapenemases

Suspension of Carbapenem susceptible strain of *Escherichia coli* ATCC 25922 was prepared and swabbed across a Mueller Hinton agar plate. Meropenem /Imipenem / Ertapenem disc (10 µg) was placed in the center of the test plate. Test isolate was streaked in a straight line from edge of the disc to the edge of the plate. After overnight incubation, positive test showed growth of *Escherichia coli* ATCC 25922 on the test streak line towards the Carbapenem disc. Negative test showed no growth of *Escherichi coli* on the test streak line (CDC 2009).

The data thus obtained was compiled, tabulated and analysed statistically.

RESULTS

The frequent use of antibiotics and even the dose and period of administration vary greatly from country to country, region to region and to some degree even locally. This has led to large differentials in the emergence of resistant strains. It is essential to study and report trends in antimicrobial resistance regularly. The emergence and dissemination of numerous types of β- lactamases as ESBLs, MBLs and AmpC enzymes among Gram negative bacterial strains pose a therapeutics challenge to the health care settings. These enzymes collectively can hydrolyze almost all β-lactum drugs which are most frequently used including Carbapenems which are called the last resort for the treatment of serious infection. A total of 427 non-duplicate MDR Gram-negative isolates were obtained from ICU patients, during the 4-year study period. The isolates were identified, and their antimicrobial resistance patterns was observed. Maximum number of isolates were identified from Medical ICU 288/427 (66.0%), followed by Surgical ICU 71/427(17%), ICU 30/ 427(7%), Intensive Coronary Care unit 16/427(4%), Neonatal ICU 12/427(3%), Pediatrics ICU 6(1%) and Burn ICU only 4(0.93%). As shown in table.1

Table 1: Location wise distribution of MDR GNB Isolates from various ICUs

ICU	No. of Samples
MICU	288(66%)
SICU	71(17%)
1CU	30(7%)
CCU	16(4%)
NICU	12(3%)
PICU	6(1%)
BICU	4(%)

Gender based distribution revealed 224/427 (52%) samples from males and 203/427 (48%) from females making male: female ratio as 1.1:1. Also, majority of isolates belonged to patients above 60 years of age followed by 31 -45years age group as shown in Table 2

Table 2: Distribution of MDR GNB isolates from ICU urine samples, according to sex and age groups(n=427)

Age	Male		Female	
	N	%	N	%
1-15yrs	10	4.5	6	3.0
16- 30yrs	19	8.4	13	6.4
31-45yrs	27	12.1	21	10.3
46-60 yrs	60	26.8	68	33.5
Above 60yrs	108	48.2	95	46.8

Most frequent GNB isolates were *Escherichi coli* 255/427 (60%) followed by *Klebsiella pneumoniae* 81/427 (19%), *Pseudomonas aeruginosa* 51/427(12%), *Acinetobacter baumannii* 14/427 (3.27%) , *Providencia rettgeri* 12/427(3%) , *Proteus spp* 8/427(2%), *Enterobacter spp* 4/427 (0.98%), *Citrobacter freundii* and *Serratia liqefaciens* group 1/427(0.123%) each in decreasing order as shown in table 3.

Table 3: Species wise distribution of MDR GNB isolates

Gram Negative Bacilli	Frequency	Percentage
Escherichia coli	255	60.0
Klebsiella pneumoniae	81	19.0
Pseudomonas aeruginosa	51	12.0
Acinetobacter baumannii	14	3.0
Providentia rettgeri	12	3.0
Proteus spp.	8	2.0
Enterobacter spp	4	1.0
Citrobacter freundii	1	0.2
Serratia liquefaciens group	1	0.2
Total	427	100.0

Among *E.coli* the highest sensitivity was seen to Amikacin (68%) followed by Cefriaxone /EDTA/ Sulbactum (66%) and least to Nalidixic acid (18%) and Ticarcillin (16%). *klebsiella pneumoniae* showed highest sensitivity to Gentamicim (65%) followed by Amoxyclav (52%) less to Ticarcillin (12%) and Cefepime (6%) *Pseudomonas aeruginosa* showed highest sensitivity to Polymyxin B (65%) and least to Ceftriaxzone (8%) *Acinetobacter baumanii* also showed maximum sensitivity (71%) to Polymyxin B but 100% resistance to Ampicillin , Ceftazidime/ Clavanate and Gentamicin

Table 4: Antimicrobial Sensitivity Pattern of Isolates

Antibiotic	***E.coli* (n=255)**	***k. pneumoniae*. (n=81)**	***P. aeruginosa* (n=51)**	***A. baumannii* (n=14)**	***p. rettgeri*(n=12)**	***Proteus spp* (n=8)**
Ampicillin	15%	15%	12%	0	0	25%
Cefixime	19%	16%	12%	14%	0	13%
Ceftazidime	24%	20%	14%	7%	0%	13%
Cefoxitin	36%	25%	41%	-	0%	38%
Ceftriaxone	18%	20%	8%	7%	0%	63%
Meropenem	68%	25%	-	-		3%
Imipenem	-%	-%	27%	-%	-%	-%
Gentamicin	58%	65%	29%	0%	0%	38%
Ciprofloxacin	19%	23%	14%	7%	0%	13%
Norfloxacin	24%	23%	18%	17%	0%	13%
Cefoperazone/Sulbactum	29%	19%	16%	29%	0%	38%
Piperacillin /Tazobactum	56%	28%	33%	14	0%	50%
Amoxicillin/Clavulanic acid	24%	52%	16%	0%	0%	13%
Nitrofurantoin	49%	17%	22%	4%	0%	13%
Colistin	49%	52%	49%	64%	0%	50%
Tigecycline	45%	47%	39%	50%	0%	25%

ESBL production was maximum detected in *Escherichia coli* 121/255(47.45%) followed by *Klebsiella pneumonia* 33/81(40.7%), *Pseudomonas aeruginosa* 6/51(11.76%), *Providencia rettgeri* 6/12 (50*%), Acinetobacter spp* 4/14(28.5) *Proteus spp* 4/8 (50%) and *Enterobacter spp.* 1(25%)

Figure 1: Screening for ESBL CAZ-ceftazidime CTX-cefotaxime CPD-cefpodoxime

Figure 2: ESBL Confirmatory Test Double disc synergy test (DDST)

AmpC production was also highest in *Escherichia coli* 72/255(28.3%) followed by *Klebsiella pneumonia* 28/81(34%), and however least production was seen in *Pseudomonas aeruginosa* 5/51(9.8%).

Figure 3: Screening result for AmpC producer showing resistant to cefoxitin(Cx)

Figure 4: Isolate showing clear distortion of the zone of inhibition indicating AmpC producers

Majority of MBL were observed in *Escherichia coli* 18/255 (7%), followed by *Klebsiella pneumoniae* 17/81(20.98%), *Pseudomonas aeruginosa* 16/51(31.37%) and least in *Acinetobacter spp* 3/14 (21.4%) as shown in table 5.

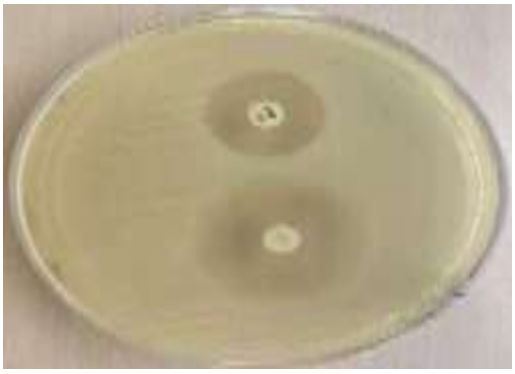

Figure 5: DPT-Strain showing ≥7mm of increase in zone of inhibition of IMP-EDTA as compared to IMP disc alone

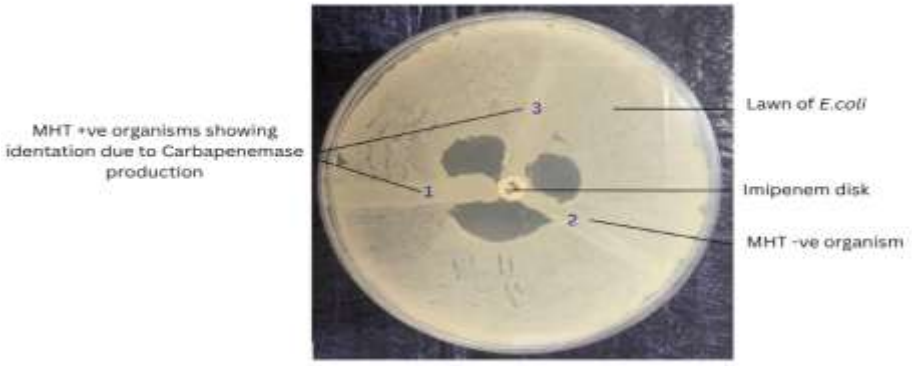

Figure 6: The MHT performed on a Muller Hinton Agar plate. (1) MHT positive result (2) MHT negative result and (3) a clinical isolate, positive result

Table: 5 Distribution of ESBL, AmpC and MBL among GNB isolates

G.N.B	N isolated	ESBL	AmpC	MBL
Escherichia. Coli	255(60%)	121(47.45%)	72(28.23%)	18(7%)
Klebsiella pneumonia	81(19%)	33(40.7%)	28(34.5%)	17(20.98%)
Pseudomonas aeruginosa	51(12%)	6(11.76%)	5(9.8%)	16(31.37%)
Acinetobacter baumannii	14(3%)	4(28.5%)	1(7.1%)	3 (21.4%)

Providencia rettgeri	12 (3%)	6(50%)		5 (41.6%)
Proteus spp.	8(2%)	4(50%)		-
Enterobacter spp	4(1%)	1(25%)	1	
Citrobacter freundii	1(0.23%)	-	-	-
Serratia liquefaciens group	1(0.23%)	-	-	-
	427	175(40.9%)	106(24.82%)	59(13.81%)

In our study, the overall MBL production was 13.81%, with the highest occurrence in *Pseudomonas aeruginosa* (31.37%), followed by *Acinetobacter spp* (21.4%), *Klebsiella pneumoniae* (20.98%) and the lowest in *E. coli* (7%).

In our study co-production was observed as 21(8.2%) , 3(1.17%) , and 50(19.6%) in *E.coli* strains E/M, A/M and E/A respectively; 5(6.17%) ,1 (1.23%), and 12(14.8%) *klebsiella pneumonia* strains E/M, A/M and E/A respectively; 5(9.8%) and 1(1.9%) *Pseudomonas aeruginosa* strains E/M and E/A respectively; 2(14.2%) and 1(7.14%) *Acinetobacter baumannii* strain A/M and E/A respectively while only 1(25%) *Enterobacter* strain had E/A.

Table :6 Co production of ESBL, AmpC, and MBL β lactamases among MDR GNB isolates

Organism	E/M	A/M	E/A
E.coli 255	21(8.2%)	3(1.17%)	50(19.6%)
Klebsiella pneumonia 81	5(6.17%)	1(1.23%)	12(14.8%)
Pseudomonas aeruginosa 51	5(9.8%)	-	1(1.9%)
Acinetobacter baumannii 14	-	2(14.2%)	1(7.14%)
Providencia rettgeri 1	-	-	
Proteus spp . 8	-	-	
Enterobacter spp 4	-	-	1(25%)
Citrobacter freundii 1	-	-	
Serratia liquefaciens group 1	-	-	-
	31(7.25%)	6(1.40%)	65(15.22%)

DISCUSSION

The emergence and dissemination of GNB in ICUs have become a major global health concern, posing a significant challenges to patient management and infection control. Critically ill patients are highly vulnerable due to invasive procedures, prolonged hospital stays, and broad-spectrum antibiotic exposure, which promote colonization and infection by resistant pathogens (Karmakar et al., 2025). This study is based on the antimicrobial resistance and multidrug resistance of the organisms isolated from ICU. Antibiotics are among most commonly prescribed drugs in ICU. Our study, conducted on 427 ICU patients at Sri Guru Ram Das Institute of Medical Sciences and Research, Vallah, Sri Amritsar and Acharya Shri Chander College of Medical Sciences and Hospital, Sidhra, Jammu over four years, provides valuable insights into the spectrum of MDR GNB, their antimicrobial resistance pattern . In our study most isolates originated from the Medical ICU, comprising 288 of 427 cases (66.0%), followed by the Surgical ICU with 71 cases (17%). These results align with prior studies, done by ,Ghanshani and Balakrishan who found maximum cases from medical ICU as 50.6%, 98% (Ghanshani et al., 2015 and Balakrishnan S et al., 2019). Most MDR GNB isolates were from males (52%) where majority of patients belonged to age group > 60yrs. These findings align with prior studies (Dumade et al., 2019 and Alebel et al., 2021). In our study, *Escherichia coli* (60%) was the most frequently isolated GNB, followed by *Klebsiella pneumonia* (19%), *Pseudomonas aeruginosa* (12%), *Acinetobacter baumanni* (3%), Providencia rettgeri (3%) and others.

These patterns align with prior research done by Pattanayak who reported *E.coli* as the leading isolate (52.7%)(Pattanayaka et al., 2013) In another study, it was reported *E. coli* (41.4%) most prevalent, followed by *Pseudomonas spp*. (40.0%), *Klebsiella spp*. (23.5%), and *Acinetobacter spp*. (29.4%) (Dumade et al., 2019). Similarly in a study done in Iran *E.coli* (76.9%) and *K. pneumoniae* (3.8%) were the most prevalent. (Hashemian et al., 2023). In our study , among the 255 *E. coli* isolates, Meropenem showed the highest sensitivity (68%), followed by CEC (66%), Amikacin (63%), sensitivity to Cephalosporins varied from 15% (Cefpodoxime) to 34% (Ceftazidime), while Penicillin-class drugs had very low sensitivity rates: 15% for Ampicillin and 16% for Ticarcillin. Similar pattern was observed in a study done (Mainai et al., 2023 and Joseb et al.,2023). In our study, *Klebsiella pneumoniae* isolates showed the highest sensitivity to Gentamicin (65%), followed by Amoxiclav and Colistin (52% each), Our results align closely with a study reported from Satara, (Patil et al., 2016). However, our findings diverge sharply from a study where, it was observed that a very high sensitivity to Cefotaxime and Ceftazidime (92% each), Cefepime (86%), Ciprofloxacin (82%), Amikacin and Amoxiclav (76% each), Cotrimoxazole, Piperacillin/Tazobactam, and Levofloxacin (73% each), Imipenem (69%), and Tigecycline (57%) was noted (Swakoli et al., 2019) In the present study, *Pseudomonas aeruginosa* isolates showed the highest susceptibility to Polymyxin B and Gentamicin (65% each), followed by Colistin (52%–44%), `Piperacillin/Tazobactam (33%), Carbapenems (25%), Tigecycline (47%), Fosfomycin (40%), and Nitrofurantoin (22%). Ticarcillin had modest activity (13%) among Penicillins. Within Cephalosporins, Cefpodoxime led with 18%, followed by Ceftazidime and Cefepime (14% each), and Cefixime (12%). Tobramycin exhibited the lowest activity (4%). These findings share trends with prior research but vary in specifics. Alebel reported very high activity for Cefepime (90%) and Imipenem (83%), moderate susceptibility to Ciprofloxacin (45%), and no activity against Ampicillin, Gentamicin, Ceftazidime, Ceftriaxone, or Tetracycline (Alebel et al., 2012). In another study a comparable profile was observed, with highest susceptibility to Tigecycline (37%), followed by Imipenem and Piperacillin/Tazobactam (18% each). In our study, *Acinetobacter spp*. exhibited the highest sensitivity to Polymyxin B (71%), followed by Colistin (64%) and Tigecycline (50%). Complete resistance was seen for Ampicillin, Amoxiclav, Amikacin, Gentamicin, and Nalidixic acid. In our study, *Providencia rettgeri* isolates exhibited resistance to most antibiotics, with partial sensitivity observed for Imipenem (50%), Fosfomycin (42%), and Ticarcillin (25%). These findings are comparable to those reported by Gangadhar, in his study where all isolates were resistant to the majority of antibiotics except Imipenem, which showed 100% sensitivity (Gangadhar et al., 2019). Similarly, in another study it was found that *P. rettgeri* was resistant to all tested antibiotics except Amoxyclav and Cotrimoxazole, both demonstrating complete sensitivity (Dumade et al., 2019). In our study, *Proteus vulgaris* showed the highest sensitivity to Cefoperazone /Sulbactam (80%), followed by Amikacin, Gentamicin, Cefoxitin, and Colistin (60% each). In our study, *Enterobacter spp.* exhibited the highest sensitivity (50%) to Piperacillin/Tazobactam, Aminoglycosides (Amikacin and Gentamicin), Norfloxacin, and Ertapenem. Conversely, all isolates demonstrated complete resistance to most Cephalosporin. These findings align partially with the study conducted by Marzieh , which reported 100% sensitivity to Amikacin, 50% sensitivity to Imipenem, and complete resistance to Cephalosporins and Ciprofloxacin (Hashemian et al., 2023)

The infections which are caused by multidrug-resistant Gram negative bacilli that produce various β lactamase enzymes have been reported with an increasing frequency in the intensive-care units and they are associated with a significant morbidity and mortality . The numerous β- lactamases are encoded either by the chromosomal genes or by the transferable genes which are located on the plasmids or the transposones .(Oberoi et al., 2013). The ESBL production in our study was (40.9%) found to be maximum as compared to the other β lactamases. Similar findings were reported in a study which was done by Bandekar et al, which showed a high prevalence of the ESBL producers (39.8%) in burn patients (Bandekar et al., 2011). A study which was done by Harakuni et al reported a high prevalence of the ESBLs (74%) in ICU patients (Harakuni et al., 2011). Laghawe et al, in his study, reported 19.67% ESBL producers (Laghawe et al., 2012). It has been proved that the prevalence of the ESBLs among the clinical isolates varies from country to country and institution to institution within the same country. In our study, the AmpC production was seen in 24.82 % isolates as compared to that in other studies that had reported a prevalence of the AmpC producers. It was 17.3% in Kolkata and 22.9% in a study which was done by Bandekar et al (Bandekar et al., 2011) in burn patients, and Bhattacharjee et al showed 22% AmpC producing *Pseudomonas aeruginosa* (Bhattacharjee et al 2008). In the index study, the MBL producers were 13.81%. Our findings were in concordance with the study which was done by Bandekar et al, who reported 15.7% MBL producers (Bandekar et al 2011) . The coexistence of different classes of β-lactamases in a single bacterial isolate may pose diagnostic and treatment challenges. The AmpC producing organisms can act as a hidden reservoir for the ESBLs. Also, the high-level expression of the AmpC β-lactamases may mask the recognition of the ESBLs and it may result in a fatal and an inappropriate antimicrobial therapy. In our study co-production was observed as 21(8.2%) , 3(1.17%) , and 50(19.6%) in *E. coli* strains E/M, A/M and E/A respectively; 5(6.17%) ,1 (1.23%), and 12(14.8%) *klebsiella pneumonia* strains E/M, A/M and E/A respectively; 5(9.8%) and 1(1.9%) *Pseudomonas aeruginosa* strains E/M and E/A respectively; 2(14.2%) and 1(7.14%) *Acinetobacter baumannii* strains ,A/M and E/A respectively while only 1(25%) *Enterobacter strain* had E/A. In the study done by (Oberai et al., 2012), the co- production of the ESBL/MBL/ AmpC β-lactamases was observed as (19.04%) . The ESBL and MBL co production was detected as (8.79%) and it was found to be maximum in *Escherichia coli* (33.34%), *Pseudomonas aeruginosa* (25%) and *Klebsiella pneumoniae* (16.67%), which is quiet different than our study. While the ESBL and the AmpC co- producers were 18 (6.59%) which is quite similar to our study and they were commonly isolated from *Escherichia coli* (50%), *Klebsiella pneumoniae* (22.23%) and *Acinetobacter baumannii* (16.67%) . The co- production of AmpC and MBL was observed as (3.67%) and it was detected in *Escherichia coli* . Our results did not match with the A/M co-production as we got the findings on higher side but we got the predominant organism as *E.coli* in our study.

CONCLUSION

Antimicrobial resistance is an escalating global public health challenge. Gram negative bacilli with diverse resistance mechanisms—such as extended-spectrum β-lactamase (ESBL), Metallo-β-lactamase (MBL) and AmpC production, and combinations of these are increasingly reported worldwide. This trend is largely the consequence of widespread and indiscriminate antimicrobial use. The problem is particularly pronounced in developing countries, where antibiotic regulation is often lacking. At the same time, the pace of new antimicrobial drug development has slowed considerably, with only a few agents introduced each year. Strict infection control practices, proper following of antibiotic policies and measures to restrict the indiscriminate use of Cephalosporins and Carbapenems in the hospital environment should be undertaken to minimize the emergence of this multiple β- lactamase producing organism whose spread would leave no other option to treat MDR Gram- negative bacterial infections. The increase in the prevalence of the AmpC, MBL and the ESBL producing isolates may be indicative of the ominous trend of more and more isolates acquiring the resistance mechanisms, thus rendering the antimicrobial armamarium ineffective.

REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941-53.
2. Mathur P, Sathishkumar K, Chaturvedi M, Das P, Sudarshan KL, Santhappan S, et al. Cancer statistics 2020: report from national cancer registry programme, India. JCO Glob Oncol. 2020;6:1063-75.
3. Global Adult Tobacco Survey India 2016-17. Ministry of Health and Family Welfare, Government of India. 2018. Available from: https://mohfw.gov.in.
4. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst. 2000;92(9):709-20.
5. Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30 Suppl 5:F55-70.
6. Huang SH, O'Sullivan B. Overview of the 8th edition TNM classification for head and neck cancer. Curr Treat Options Oncol. 2017;18(7):40.
7. Hobbs CG, Sterne JA, Bailey M, Heyderman RS, Birchall MA, Thomas SJ. Human papillomavirus and head and neck cancer: a systematic review and meta-analysis. Clin Otolaryngol. 2006;31(4):259-66.
8. Koskinen WJ, Chen RW, Leivo I, Makitie A, Back L, Kontio R, et al. Prevalence and physical status of human papillomavirus in squamous cell carcinomas of the head and neck. Int J Cancer. 2003;107(3):401-6.
9. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? Cancer. 2007;110(7):1429-35.
10. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer. 2003;3(10):733-44.
11. Yang D, Shi Y, Tang Y, Ni J, Xiao B, Peng P, et al. Effect of HPV infection on the occurrence and development of laryngeal cancer: a review. J Cancer. 2019;10(19):4455-62.
12. Vande Pol SB, Klingelhutz AJ. Papillomavirus E6 oncoproteins. Virology. 2013;445(1-2):115-37.
13. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science. 2011;333(6046):1154-7.
14. Westra WH. Detection of human papillomavirus (HPV) in clinical samples: evolving methods and strategies for the accurate determination of HPV status of head and neck carcinomas. Oral Oncol. 2014;50(9):771-9.
15. Lewis JS Jr, Beadle B, Bishop JA, Chernock RD, Colasacco C, Lacchetti C, et al. Human papillomavirus testing in head and neck carcinomas: guideline from the College of American Pathologists. Arch Pathol Lab Med. 2018;142(5):559-97.
16. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev. 2005;14(2):467-75.
17. Gama RR, Carvalho AL, Longatto Filho A, Scorsato AP, Lopez RVM, Rautava J, et al. Detection of human papillomavirus in laryngeal squamous cell carcinoma: systematic review and meta-analysis. Laryngoscope. 2016;126(4):885-93.
18. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12-9.
19. Castellsague X, Alemany L, Quer M, Halec G, Quiros B, Tous S, et al. HPV involvement in head and neck cancers: comprehensive assessment of biomarkers in 3680 patients. J Natl Cancer Inst. 2016;108(6):djv403.
20. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene. 2002;21(48):7435-51.
21. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell. 1990;63(6):1129-36.
22. Syrjanen S, Syrjanen K, Mantyjarvi R, Collan Y, Karja J. Human papillomavirus (HPV) DNA sequences demonstrated by in situ DNA hybridization in serial sections of some squamous cell carcinomas of the larynx. ORL J Otorhinolaryngol Relat Spec. 1987;49(3):175-86.
23. Gallus R, Meloni F, Rizzo D, Pichi B, Manciocco V, Cristalli G, et al. Prevalence of HPV infection and p16INK4a overexpression in laryngeal squamous cell carcinoma. Vaccines (Basel). 2022;10(2):204.
24. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst. 2008;100(4):261-9.

25. Colevas AD, Yom SS, Pfister DG, Spencer S, Adelstein D, Adkins D, et al. NCCN guidelines insights: head and neck cancers, version 1.2018. J Natl Compr Canc Netw. 2018;16(5):479-90.
26. Boscolo-Rizzo P, Tirelli G, Fuson R, Lupato V, Spinato G, Gava A. Prognosis of supraglottic carcinoma. Eur Arch Otorhinolaryngol. 2008;265(12):1447-54.
27. Yugawa T, Kiyono T. Molecular mechanisms of cervical carcinogenesis by high-risk human papillomaviruses: novel functions of E6 and E7 oncoproteins. Rev Med Virol. 2009;19(2):97-113.
28. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010;363(1):24-35.
29. Mehanna H, Beech T, Nicholson T, El-Hariry I, McConkey C, Paleri V, et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer—systematic review and meta-analysis of trends by time and region. Head Neck. 2013;35(5):747-55.
30. Agalliu I, Gapstur S, Chen Z, Wang T, Anderson RL, Teras L, et al. Associations of oral alpha-, beta-, and gamma-human papillomavirus types with risk of incident head and neck cancers. JAMA Oncol. 2016;2(5):599-606.
31. Schache AG, Liloglou T, Risk JM, Filia A, Jones TM, Sheard J, et al. Evaluation of human papilloma virus diagnostic testing in oropharyngeal squamous cell carcinoma: sensitivity, specificity, and prognostic discrimination. Clin Cancer Res. 2011;17(19):6262-71.
32. Lewis JS Jr, Thorstad WL, Chernock RD, Haughey BH, Yip JH, Zhang Q, et al. p16 positive oropharyngeal squamous cell carcinoma: an entity with a favorable prognosis regardless of tumor HPV status. Am J Surg Pathol. 2010;34(8):1088-96.
33. Feller L, Khammissa RA, Chandran R, Altini M, Lemmer J. Tobacco smoking-associated downregulation of Langerhans cell numbers and function: possible link to oral carcinogenesis. Head Face Med. 2014;10:41.
34. Morshed K. Association between human papillomavirus infection and laryngeal squamous cell carcinoma. J Med Virol. 2010;82(6):1017-23.
35. Morshed K, Polz-Dacewicz M, Szymanski M, Polz D. Short-fragment PCR assay for highly sensitive broad-spectrum detection of human papillomaviruses in laryngeal squamous cell carcinoma and normal mucosa: clinicopathological evaluation. Eur Arch Otorhinolaryngol. 2008;265 Suppl 1:S89-96.
36. National Programme for Non-Communicable Diseases. Operational Guidelines. Ministry of Health and Family Welfare, Government of India. 2020. Available from: https://mohfw.gov.in/npncd.