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Detection of drug resistance pattern among Gram-negative isolates

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Abstract

Both Gram-positive and Gram-negative bacteria are one of the main causes of infections in humans. Now a day, antimicrobial resistance (AMR) is considered as main public health threat, also AMR bacteria in different hospital wards are increasing significantly. Increasing number of extended spectrum beta-lactamase (ESBL) producers has reduced the treatment options which resulted in emergence of multidrug resistant strains, treatment failure and hence increased mortality. To know the drug resistance pattern among gram negative isolates and detection of ESBL production. A retrospective study of all gram negative isolates was conducted. Total of 177 isolates were isolated from various clinical samples. They were processed and identified by standard Microbiological procedures. The antibiotics susceptibility testing was performed by Kirby- Bauer disc diffusion method using CLSI guidelines. ESBL was detected by combined disc test using ceftazidime (30µg) alone and in combination with clavulanic acid disc. Of 285 samples processed, 177 Gram-negative isolates were isolated which includes *E. coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp., *and Acinetobacter* spp. Males 104 (58.8%) were commonly involved as compared to females 73 (41.2%).Of 177 isolates tested,90(50.8%) are from urine sample, 83(46.9%) from pus sample and 4(2.3%) from blood sample. ESBL was detected in 92 (52%) isolates. They showed least resistance to amikacin 36 (20.3%), piperacillin-tazobactam 30 (16.9%) and meropenem 10 (5.6%). Our study showed most of the isolates was resistant to commonly used penicillin and cephalosporin group of antibiotics and also showed 52% prevalence of ESBL producers. Judicious use of antibiotics, detection and reporting of beta-lactamase enzymes helps in combating spread of MDR bacteria and also helps in appropriate treatment.

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Introduction

Many microorganisms now are unfriendly to human beings who are responsible for infections and diseases (Reta *et al.*, 2019). One of the important public health issues are infection and antibiotic resistance (Veena Kumari *et al.*, 2007). Gram negative bacteria are the commonest cause for most of the infections and are generally resistant to antibiotics. Recently gram negative bacteria resistant to most of the antibiotic groups have become more prevalent and are of concern in treatment of infections (Ullah *et al.*, 2012). The rate of survival from infections is impacted majorly by discovery of antimicrobial agents. However, increase in the prevalence of drug resistant microorganisms and the changing patterns of antimicrobial resistance demanded need for new antibacterial agents. Antimicrobial resistance is a well-known clinical and public health problem (Lamichhane *et al.*, 2014).

According to the World Health Organization (WHO), antimicrobial resistance (AMR) is a major problem worldwide in antimicrobial therapy. It is a global problem caused mainly by overuse and misuse of antimicrobial agent. AMR leads to prolonged illness, longer hospital stays, higher medical costs, and increased risk of death.

AMR constitutes a continuously growing threat to the effective treatment of microbial infections. However, the status of impact of AMR on the health of people in community and in hospitalized patients and on financial burden experienced by health care systems in the management of infections caused by drug resistant microorganisms are still uncertain (Elbadawi *et al.*, 2019). Approximately about two million are infected and 23,000 deaths results due to infection with drug resistant microorganisms each year. The health care crisis is fueled by multidrug-resistant Gram-negative organisms, including extended spectrum β lactamases (ESBL), metallobetalactamase (MBL) and AmpC beta-lactamase (Walker *et al.,* 2019). In general; there is direct association between mortality and infections caused by multi-drug resistant Gram-negative bacteria (GNB). In addition, infections with multi-drug resistance GNB lead to less desirable outcomes, including longer hospital stays and utmost cost of hospitalization (Afifi *et al.*, 2015). Now, existence of "extreme drug resistance" in GNB that are resistant to first line and second line antibiotics are increasing creating a challenge for clinicians in the management of infections (Sandhu *et al.*, 2019). Hence, the study was conducted to know the drug resistance pattern among Gram-negative isolates and detection of ESBL production.

Materials and methods

Retrospective study was conducted at District hospital Microbiology laboratory attached to Chamarajanagar Institute of Medical Sciences for duration of 1 year from July 2018 to June 2019. All gram negative bacteria isolated from various clinical samples (pus, blood, urine) were included. Data of age, gender, culture and sensitivity results were collected from Microbiology Laboratory registers. Isolates were processed and identified by standard Microbiological procedures (Collee *et al.*, 1996). The antibiotic susceptibility testing was performed by Kirby- Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI, 2019).

The following antibiotics were tested: amikacin (30µg), ampicillin (10µg), meropenem (10µg), gentamicin (10µg), ciprofloxacin (10µg), cotrimoxazole (1.25/23.75µg), cefepime (30µg), cefotaxime (30µg), cefuroxime (30µg), piperacillin-tazobactam (100/10µg), ceftazidime+clavulanic acid (30µg/10µg). Resistance data were interpreted according to Clinical laboratory Standards Institute (CLSI, 2019) guidelines.

Detection of ESBL

ESBL was detected phenotypically by combined disk test using ceftazidime (30µg) alone and in combination with clavulanic acid. Test organisms were inoculated onto Mueller Hinton agar by lawn culture. The ceftazidime disk alone and ceftazidime in combination with clavulanic acid (Caz + Cac, 30/10µg) were placed. An increase

of ≥ 5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered as the tested organism was ESBL producer.

Data analysis

Data analysis was done using MS Excel.

Ethical considerations

Ethical clearance was obtained from the Institutional Ethical clearance committee of Chamarajanagar Institute of medical sciences, Chamarajanagar.

Results

Of 285 different clinical samples processed, 177 Gram negative isolates were isolated. Of these 177 isolates, 104(58.8%) isolates were from males and 73(41.2%) were from females as shown in Table 1.

Table 1. Gender distribution of Gram-negative isolates

Organisms	Male (%)	Female (%)
Escherichia coli	36 (34.6)	40 (54.7)
Klebsiella pneumoniae	15 (14.4)	11 (15)
Pseudomonas aeruginosa	18 (17.3)	11 (15)
Proteus spp.	20 (19.2)	03 (4.1)
Enterobacter spp.	09 (8.6)	04 (5.4)
Citrobacter spp.	04 (3.8)	01 (1.3)
Acinetobacter spp.	02 (1.9)	03 (4.1)
Total (n = 177)	104 (100)	73 (100)

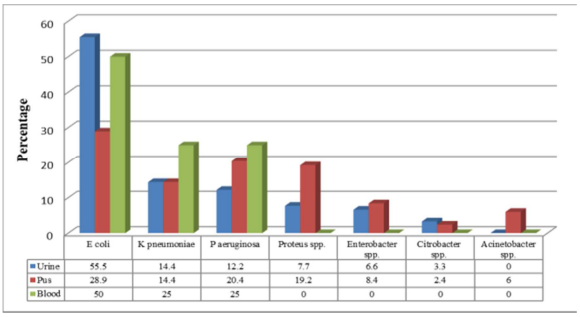

Fig. 1. Different Gram-negative isolates from different clinical samples

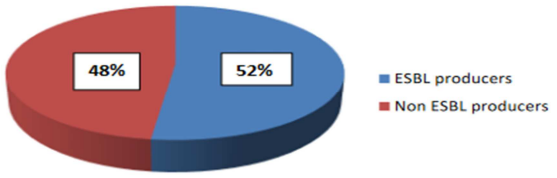

Fig. 2. Percentage of ESBL and Non ESBL producers

Table 2. No. of ESBL producers among different Gram-negative isolates

Organisms	Total isolates No. (%)	ESBL producers No. (%)
Escherichia coli	76 (42)	49 (53.2)
Klebsiellapneumoniae	26 (14.6)	15 (16.3)
Pseudomonas aeruginosa	29(16.3)	10 (10.8)
Proteus spp.	23 (12.9)	03 (3.2)
Enterobacter spp.	13(7.3)	08 (8.6)
Citrobacter spp.	05 (2.8)	04 (4.3)
Acinetobacter spp.	05 (2.8)	03 (3.2)
Total	177(100)	92 (100)

Table 3. Antibiotic resistance pattern of all Gram-negative isolates

Organism	AK	AMP	MRP	GEN	CIP	COT	CPM	CTX	CXM	PIT	CAZ	CAC
Escherichia coli	14 (7.9)	73 (41.2)	07 (3.9)	31 (17.5)	50 (28.2)	54 (30.5)	58 (32.7)	64 (36.1)	52 (29.3)	13 (7.3)	49 (27.6)	41 (23.1)
Klebsiella pneumoniae	04 (2.2)	---	00	06 (3.3)	08 (4.5)	12 (6.7)	17 (9.6)	19 (10.7)	18 (10.1)	11 (6.2)	15 (8.4)	14 (7.9)
Pseudomonas aeruginosa	03 (1.6)	---	03 (1.6)	09 (5)	09 (5)	---	20 (11.2)	16 (9)	24 (13.5)	04 (2.2)	10 (5.6)	09 (5)
Proteus spp.	09 (5)	13 (7.3)	00	07 (3.9)	12 (6.7)	15 (8.4)	09 (5)	06 (3.3)	09 (5)	00	03 (1.6)	02 (1.1)
Enterobacter spp.	01 (0.5)	13 (7.3)	00	06 (3.3)	03 (1.6)	04 (2.2)	09 (5)	09 (5)	10 (5.6)	01 (0.5)	08 (4.5)	06 (3.3)
Citrobacter spp.	03 (1.6)	---	00	00	01 (0.5)	03 (1.6)	04 (2.2)	03 (1.6)	02 (1.1)	00	04 (2.2)	03 (1.6)
Acinetobacter spp.	02 (1.1)	---	00	03 (1.6)	02 (1.1)	03 (1.6)	03 (1.6)	03 (1.6)	00	01 (0.5)	03 (1.6)	03 (1.6)
Total n=177	36 (20.3)	99 (55.9)	10 (5.6)	62 (35)	85 (48)	91 (51.4)	120 (67.7)	120 (67.7)	115 (64.9)	30 (16.9)	92 (51.9)	78 (44)

AK – Amikacin, AMP – Ampicillin, MRP –Meropenem, GEN – Gentamicin, CIP – Ciprofloxacin, COT – Cotrimoxazole, CPM – Cefepime, CTX – Cefatoxime, CXM – Cefuroxime,PIT – Piperacillin/Tazobactum, CAZ – Ceftazidime, CAC –Clavulanic acid.

Fig. 1 shows distributions of different Gram-negative isolates in different samples. Majority of gram negative isolates were found in urine 90(50.8%) followed by pus 83 (46.9%) and blood 4 (2.3%). Fig. 2 shows 92 (52%) isolates were ESBL producers. Maximum number of ESBL producers is seen in *E. coli* followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Citrobacter* spp., *Enterobacter* spp*., Acinetobacter* spp. and *Proteus* spp. as shown in Table 2. Table 3 shows that Gram-negative isolates showed least resistance toamikacin 36 (20.3%), piperacillin-tazobactam 30 (16.9%) and meropenem 10 (5.6%).

Discussion

Infections caused by these resistant organisms are responsible for treatment failure, prolonged illness and a risk of morbidity and mortality. Overuse and misuse of antimicrobial agents is the most common cause of development of acquired resistance (Jose *et al.*, 2017). Production of Beta-lactamase enzyme (in both gram positive and gram negative bacteria) is the most common mechanism for development of antimicrobial resistance. Although the prevalence of ESBL producer varies from country to country, it is more in Asia (Saxena *et al.*, 2019).

Our study showed majority of infection found in males i.e., 58.7% compared to females 41.2% which was in concordance with studies done by Azimi *et al.* (2019) and Shehkar *et al.* (2016) which showed 55% for male and 45% for females and 55.5% for male and 44.5% for females respectively. Maximum gram negative isolates in our study were from urine (50.84%) samples followed by pus (46.89%) and blood (2.25%) with *E. coli* being most common isolate which is similar with studies done by Batchoun *et al.* (2019) which showed higher distribution in urine (56%) and Zaman *et al.* (2015) study showed higher distribution of gram negative isolates in urine (43.75%) and least distribution in blood (6.25%). *Escherichia coli and Klebsiella* species are members of family Enterobacteriaceae, which are most frequently associated with various systemic infections particularly as opportunistic pathogens in clinical settings (Phamba *et al.,* 2017).

Present study showed prevalence of ESBL was 52 % which correlated with the study done by Moses *et al.* (2014) which showed prevalence of 50.8% using genotypic methods. Majority of the isolates carried the *bla*CTX-M, followed by the blaTEM and blaSHV. ESBL production was highest in *E. coli* (53.2%) and *Klebsiella pneumoniae* (16.3%) which correlates with study of Khan *et al.* (2015). Increasing resistance to commonly used antibiotics such asampicillin, ciprofloxacin and tetracyclinehas caused considerable alarm. Our study showed most of the gram negative isolates were least resistant to amikacin (20.3%), piperacillin-tazobactam (16.9%) and meropenem (5.6%). Similar findings have been reported by Shilpakar *et al.* (2021). The unregulated use of antibiotics may explain the highest resistance to penicillin and cephalosporin group of antibiotics. Carbapenem is the most active antibacterial agent for ESBL-producing Gram negative bacteria as it is not affected by ESBL (Ogefere *et al.*, 2015). Their overuse is likely to increase resistance (e.g. carbapenemase production) to these active antibiotics. It is therefore important to detect resistance mechanisms in order to justify use of carbapenems (Affolabi *et al.*, 2017).

MDR Gram-negative bacteria are common health care associated pathogens. Thus it is important to perform routine detection of ESBLs and other β-lactamases in tertiary care hospitals. Infection control measures, hygiene guidelines; appropriate antibiotic policies that control the widespread use of antibiotics are required decrease problem of the emergence of MDR ESBL producing Gram-negative bacteria (Ghimire *et al.*, 2017).

Conclusion

Overuse and inappropriate use of antibiotics is favoring spread of multidrug resistant (MDR) bacteria. Our study showed most of the isolates was resistant to commonly used penicillin and cephalosporin group of antibiotics and also showed 52% prevalence of ESBL producers. ESBL producing bacteria are associated with clinical and treatment failure. Judicious use of antibiotics, detection and reporting of beta-lactamase enzymes helps in combating spread of MDR bacteria and also helps in appropriate treatment.

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