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RESEARCH ARTICLE

ANTIBIOTIC RESISTANCE PATTERN OF GRAM NEGATIVE BACTERIAL ISOLATES FROM THE CLINICAL SAMPLES FROM CRITICAL CARE UNITS OF A TERTIARY CARE CENTRE

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ABSTRACT: Antimicrobial resistance (AMR) is a major issue especially in patients admitted in Intensive care units. Irrational overuse and misuse of antibacterial drugs has made the pathogens resistant to them. In eastern countries, infections in ICU are caused mainly by Gram negative pathogens, which are MDR. Hence, this study was conducted to study the gram negative pathogens causing ICU infections in our centre and their drug resistance pattern. **Material and Methods**: It was a prospective study done for four months (Dec 2015-March 2016) in different ICU of the tertiary care hospital. All patients admitted for more than 48 hours in ICU and having symptoms of infections were included in the study. The relevant sample was collected and preceded as per Standard microbiological procedure and Antimicrobial susceptibility test was done by Kirby Bauer's disc diffusion method as per CLSI guidelines. **Results:** During the study period, 327 patients were admitted in ICUs, of which 173 were admitted for more than 2 days. A total of 1013 samples were received from ICU, only 245 samples showed significant bacterial growth. Majority of the isolates were Gram negative pathogens accounting for 209 (73.8%) of the 283 total pathogens isolated. Respiratory infection and Urinary tract infections, were the commonest infections accounting for around 25% each. *Klebsiella* species and *Acinetobacter* species were the commonest Gram negative pathogen isolated. *Klebsiella* species and *Escherichia coli* were resistant to most of the commonly used drugs like aminoglycosides, 3rd Generation cephalsporins (3GC) and β lactam – β lactamase Inhibitor (BL-BLI) combination, with only resort being carbapenems. But, in case of *Acinetobacter* species, 92% of the isolates were resistant to carbapenems. **Conclusion:** The infections in patients admitted in ICU are predominantly caused by MDR Gram negative bacteria. The commonly administered antibacterial drugs in ICU like aminoglycosides, 3GC and BL-BLI are not effective in combating these pathogens. It's high time to implement antibiotic stewardship programme to combat this menace of drug resistance.

KEY WORDS: ICU infections, Nosocomial infections, MDR-GNB

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INTRODUCTION:

Antimicrobial resistance (AMR) is a global issue; due to lack of newer antimicrobial drugs, we are heading to pre-antibiotic era. The multi-drug resistant (MDR) bacteria emerge mainly from the hospitals, especially from ICU, where the antimicrobial pressure is very high[1]. The irrational overuse and abuse of high end antimicrobial drugs make these pathogens multi-drug resistant. The most vulnerable patients are admitted in ICU and are treated empirically with antimicrobial drugs. Depending on the age, co-morbid conditions, length of ICU stay, severity of illness and invasive devices, these patients acquire HAI with MDR pathogens [2]. Eventhough, the ICU beds account for 5-10% of total beds in hospital, they account for 10-25% of the health care cost [3]. These HAI are caused mainly by the endogenous flora of the patients which are MDR due to prior exposure to antimicrobial drugs. To effectively start an appropriate empirical antimicrobial therapy, each intensivist needs to know the common pathogens of their ICU and their antibiotic resistance pattern antibiogram. In developing countries, Gram negative pathogens accounts for more than 60% of the all pathogens causing HAI in ICU. The aim of the study was to identify the frequency of predominantly isolated Gram negative bacterial isolates and their drug resistance patterns for the patients admitted in different ICUs (MICU, BICU, SICU and ICCU) at tertiary care centre.

MATERIALS AND METHODS:

This was a prospective study conducted at the different Intensive Care Units (ICUs) of 1250 bedded, tertiary care teaching hospital, conducted from December 2015 to March 2016, after approval from Institutional Ethics Committee. The Centre for Disease Control and Prevention (CDC) defines ICU associated infections are those that occur after 48 hours of ICU admission or within 48 hours after transfer from an ICU. All patients admitted in any of the four ICUs (MICU, SICU, BICU and ICCU) and who were clinically suspected of having acquired any infection after 48 hours of admission to the ICUs were included. Few clinical signs and symptoms suggestive of infections are as follows: fever>38°C, Leucocytosis >10,000/mm^3, New infiltrates on chest X ray, Persistent Tracheal aspirates/secretions, Turbid urine, Suprapubic tenderness, Dysuria, Burning micturition, etc. The inclusion criterion was to study the gram negative bacteria isolated from patients who were admitted in ICUs of the hospital. Patients showing clinical signs of infection on or prior to admission or transfer to the ICUs and patients with Gram positive bacterial infections were excluded from the study. Depending on the clinical suspicion samples were collected from the patients and transported immediately to Clinical microbiology lab. The samples were inoculated into 5% Sheep Blood Agar, Mac-Conkey Agar and chocolate agar, incubated aerobically and in candle jar, respectively, at 37^{0}C. The gram negative isolates were identified by standard biochemical reactions. The following antibiotic disc were used for antibiotic sensitivity testing according Kirby bauer's disc diffusion method on Mueller Hinton's agar : Ampicillin (10µg), Amoxicillin/ clavulanic acid (20/10µg), Ceftriaxone (30µg) / Ceftazidime (30µg), Cotrimoxazole (25µg), Gentamycin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Doxycycline (30µg), Tigecycline(15µg), Piperacillin/Tazobactam (100/10µg), Imipenem (10µg), Meropenem (10µg). Zone of diameter was measured and interpreted as per the Clinical and Laboratory Standard Institute guidelines[4]. The ATCC *Escherichia coli* 25922 and *Staphylococcus aureus* 25923 were used for quality control.

RESULT:

Table 1. Distribution of the samples according to Intensive care units

	Distribution of Samples Based on ICUs	Frequency of Growth in ICUs
MICU	411 (40.6)	124 (50.6)
BICU	85 (8.4)	54 (22.0)
ICCU	82 (8.1)	41 (16.7)
SICU	435 (42.9)	26 (10.6)
Total	1013	245(24.2%)

Table 2: Profile of samples from ICU and Rate of Positive Culture

Sample	Total Samples (n = 1013) (%)	Samples with Growth (n = 245) (%)	Samples with growth of Gram negative Isolates (n =171) (%)
Blood	**591 (58.3)**	**67(27.3)**	35(20.5)
Urine	**143 (14.1)**	53 (21.6)	**40 (23.4)**
CSF	66 (6.5)	NIL	NIL
Sputum	51 (5.0)	22 (8.1)	16 (9.4)
Endotracheal aspirate	51 (5.0)	31 (12.7)	25 (14.6)
Pus	32 (3.2)	31 (12.7)	22 (12.9)
Wound swab	30 (2.1)	30 (12.3)	22 (12.9)
CVC tip	11 (1.1)	5 (2.0)	5 (2.9)

Table 3. Pattern of Gram negative Bacteria Isolated from samples Different ICUs

	Frequency (%) n=209	MICU (%) n= 101	BICU (%) n=61	ICCU (%) n=26	SICU (%) n=21
Klebsiella pneumoniae	**54 (25.8)**	20 (19.8)	18 (29.5)	**10 (38.5)**	**6 (28.6)**
Acinetobacter spp.	49 (23.4)	25 (24.7)	**20 (32.8)**	1 (3.8)	3 (14.3)
Escherichia coli	42 (20.1)	**28 (27.7)**	2 (3.3)	7 (26.9)	5 (23.8)
Pseudomonas aeruginosa	39 (18.7)	15 (14.8)	18 (29.5)	5 (19.2)	1 (4.8)
Citrobacter spp.	13 (6.2)	6 (5.9)	2 (3.3)	1 (3.8)	4 (19.0)
Klebsiella oxytoca	4 (1.9)	2 (1.9)	_	_	2 (9.5)
Enterobacter spp.	3 (1.4)	3 (2.9)	_	_	_
Proteus mirabilis	2 (0.9)	1 (0.9)	1 (1.6)	_	_
Proteus vulgaris	2 (0.9)	1 (0.9)	_	1(3.8)	_
Chryseobacterium sp	1 (0.5)	-	-	1(3.8)	-

Table 4: Pattern of Gram negative Bacteria Isolated from Different Samples

		Sample collected from						
Organisms	Frequency (%) n=209	Blood (n= 38) (%)	Urine (n=45) (%)	ETA (n=31) (%)	Sputum (n=18) (%)	Pus (n=34) (%)	W.swab (n=32) (%)	CVC tip (n=5) (%)
Klebsiella pneumoniae	54 (25.8)	12(31.5)	7(15.5)	8 (25.8)	7 (38.9)	8 (23.6)	10 (31.3)	1 (20)
Acinetobacter spp.	49 (23.4)	7(18.4)	2 (4.4)	10 (32.2)	7(38.9)	11(32.4)	9 (28.1)	2 (40)
Escherichia coli	42 (20.1)	10 (26.3)	18(40)	4 (12.9)	1 (5.6)	5 (14.7)	1 (3.1)	1 (20)
Pseudomonasaeruginosa	39 (18.7)	6 (15.8)	4 (8.9)	6(19.4)	3 (16.7)	9 (26.5)	9 (28.1)	1 (20)
Citrobacter spp.	13 (6.2)	_	7 (15.5)	2 (6.4)	_	_	2 (6.4)	_
Klebsiella oxytoca	4 (1.9)	_	4 (8.9)	-	_	_	_	_
Enterobacter spp.	3 (1.4)	2 (5.3)	_	1 (3.2)	_	_	_	_
Proteus mirabilis	2 (0.9)	1 (2.2)	_	_	_	_	1 (3.1)	_
Proteus vulgaris	2 (0.9)	1 (2.2)	_	_	_	1 (2.9)	_	_
Chryseobacterium sp	1 (0.5)	1 (2.2)	_	_	_	_	_	_

During the study period (December 1, 2015 to March 31, 2016), a total of 327 patients got in the ICUs . Of which 173 were admitted for more than 2 days.Out of 1013 samples analysed, majority of the sample was received from SICU 435 (42.9%) followed by MICU 411 (40.6 %) (Table 1). The most common sample received was Blood 591 (58.34%), followed by urine 143 (14.11%) as shown in table 2. Only 245(24.2%) patient sample had significant bacterial growth, from which a total of 283 different isolates were obtained. Majority of the positive sample were from MICU 124 (50.6%) (Table 1).Out of 245 samples, 207 (84.5%) showed single isolates, whereas 38 (15.5%) showed more than one (up to three) isolates. Among 283 pathogens isolated, 209 (73.8%) were Gram negative pathogens. Among the total 171 samples growing Gram negative pathogens, most common was Lower respiratory tract sample (Sputum and ETA, 41, 24%), followed Urine (40, 23.4%) and Blood 35 (20.5%) (Table 2).

Among Gram negative pathogens isolated , the most common was *K. pneumoniae* 54 (25.8%) followed by *Acinetobacter* spp 49,(23.4%) (Table 3) But the commonest isolates varied among the ICUs. In MICU, *E.coli* 28 (27.723%) was predominantly isolated, whereas in BICU, ICCU and SICU had *Acinetobacter* sp 18(29.5%), *K.pneumoniae* 10 (38.5%) and *K.pneumoniae* 6(28.6%), respectively (Table 3). Among the 38 pathogens isolated from blood, *K.pneumoniae* 12 (31.5%) was the commonest, followed by *E.coli* 10 (26.3%). Among the 45 positive urine samples , *E.coli* (18, 40%) was the most common . In respiratory samples, i.e., ETA and Sputum, *Acinetobacter* sp (17/49) was predominant pathogen.. *K.pneumoniae* was the leading causing of wound infection, among the Gram negative bacteria in the ICUs (Table 4).

The drug resistance pattern among the pathogens varied individually. *K.pneumoniae* being the most common isolate was resistant to frequently used antibiotics like 3GC, aminoglycosides and β lactam – β lactamase Inhibitor combination. Only carbapenems were promising. *Acinetobacter* spp was most difficult pathogen to treat as they showed 98% resistance to Ampicillin, Ceftriazone, 96% resistance to Ciprofloxacin and Piperacillin/ Tazobactum, even carbapenem resistance in 92% of the isolates. *E.coli* also had similar resistant pattern, but β lactam – β lactamase Inhibitor combination and carbapenems were effective in more than 60% isolates.

DISCUSSION:

In a hospital, ICU contributes for more than 20% nosocomial infections, worldwide[5]. The mortality in patients with nosocomial infection in ICU can be as high as 50-60% [6]. In a multi-national Extended Prevalence of Infection in Intensive Care (EPIC II) study, which included 13,796 adult patients, a point prevalence of 51% of sepsis was reported [7]. These nosocomial infections in ICU of middle and low income countries are predominantly caused by MDR -GNB and they are at alarming rate. The factors contributing for these infections may vary among ICUs and type of the patients admitted in these ICUs.

The annual report 2017 by AMR Surveillance Network, ICMR has studied 45,930 isolates from various parts of the country has reported Gram negative pathogens as the major share of the isolates. They reported blood (25%) as the major sample from ICU, followed by Lower respiratory tract and pus (16% each) [8]. Pneumonia was the most common type HAI in the ICU of a hospital in South India, UTI and blood stream infections had lower incidence[9]. Similarly, teaching hospital of Nepal had pneumonia (16%), BSI (5.7%) and UTI (3.9%) in decreasing order of incidence of HAI in their ICU (Parajuli 2017) [10].

During the study period, pneumonia (41, 24%) was observed as the commonest HAI followed by UTI

(40, 23.4%) and BSI (20.5%), similar to study conducted in Puducherry and Nepal. *E.coli* was the most common isolate constituting for 46% followed by *Klebsiella pneumoniae* (42%) in the nationwide study (AMR, ICMR 2017)[8]. Similar observation was made by Infection control and prevention specialists at Puduchery, who had *Acinetobacter* spp, *Klebsiella* spp and *E.coli* as the common gram negative pathogens in their ICU [9]. It was observed that *Klebsiella pneumonaie* was the commonest Gram negative pathogen causing HAI in our ICUs similar to reports from ICMR. However, the microbial pathogens causing infection in ICU can differ from various ICUs in a same hospital depending on the type of the patients admitted, as we have observed (Table 3). In our ICUs, BSI was common with *Klebsiella pneumonaie*, but UTI was mainly caused by *E.coli. Acinetobacter* sp was reported to be the pathogen causing nosocomial pneumonia in our ICU (Table 4). Among the Enterobacteriaecae, more than 70% of the isolates were resistant to β lactam group of the antibiotics, aminoglycoside resistance varied between 45-65% and fluoroquinolone resistance around 55%. The most common drugs like β lactum- β lactum inhibitors combination and carbapenem resistance accounted for 60% and 50%, respectively. The non-fermentors, *Acinetobacter* spp isolates were resistant to almost all class of antibiotics including carbapenems upto 90% similar to ICMR 2017 report [8]. So, our ICU has similar pathogens that cause HAI at various ICU nationally. We have not isolated other non-fermentors like Burkholderia cepacia, *Stenotrophomonas maltophilia* in our ICU.

CONCLUSION:

The patients admitted in ICU have high chance of infections with MDR pathogens. These pathogens may have varying antimicrobial resistance pattern, differing from ICU to ICU. In the present study, *K.pneumoniae, Acinetobacter* spp., *E.coli* and *P.aeruginosa* were the most frequently isolated pathogens in ICU patients in this hospital. Majority of the isolates of *K. pneumoniae*, *Acinetobacter spp*, *E.coli* and *P.aeruginosa* are resistant to ampicillin, cephalosporins and quinolones as compared to aminoglycosides and meropenem, similar to national report. The data emphasizes that appropriate antibiotic utilization in ICU is crucial not only in ensuring an optimal outcome, but also in preventing the emergence of multi drug resistance bacteria.

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