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INCIDENCE OF DRUG RESISTANCE AMONG BACTERIAL ISOLATES OBTAINED IN A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

Introduction: Infections caused by Gram negative bacteria is major health threat to patients in intensive care units and to patients with some comorbidity. Constant surveillance of drug-resistance among the different bacterial pathogens is important in prevention and management of infections caused by these pathogens. Such type of study also help clinicians to prescribe correct drug regimen to the patients suffering from these infections which may be primary, secondary or nosocomial, reducing the morbidity, mortality and hospitalization period. The present study is undertaken to study the drug susceptibility pattern and its incidence among the different bacterial pathogens isolated from different clinical samples obtained at Tertiary Care Hospital.

Materials and Methods: The samples received from different clinical units were subjected to bacteriological investigations using standard microbiological procedures. The isolated pure cultures of bacterial pathogens were subjected to antibiotic susceptibility testing as per the CLSI guidelines of 2016. The data of antibiotic susceptibility of each isolated pathogens were tabulated and prevalence of each drug resistance and multi-drug resistance were determined to study the trends of drug resistance among these pathogens.

Results: The incidence of infections caused by Gram negative bacteria was found to be 74.67% among all the isolates and that of Gram positive bacterial infections was found to be 25.33% respectively. *Esch.coli* (37.32%) was the most frequently isolated Gram negative bacterium followed by *Ps. aeruginosa, Klebsiella spp., Acinetobacter spp*. and *Proteus spp*.in the descending order among all the isolates of Gram positive and Gram negative isolates.

Conclusion: The incidence of infections caused by Gram negative bacteria was higher than those caused by Gram positive bacteria. *Esch.coli* was the most common pathogen among the Gram negative bacterial isolates whereas *Staph.aureus* was the most common pathogen among all the Gram positive bacterial isolates. All the bacterial isolates showed 100% multi-drug resistance and the incidence of drug resistance to second generation penicillin and cephalosporins were found to be remarkably higher among all the bacterial isolates.

KEYWORDS

Esch.coli, Staph.aureus, Incidence of drug-resistance, Multi-drug resistance

INTRODUCTION

The bacterial infections in patients with co-morbidity is matter of concern for health professionals. Moreover the antimicrobial resistance among the Gram negative bacteria is more common in India and one of the important contributing factors responsible for high mortality, extended hospital stay, high treatment cost among the patients [2, 3, 5, 15]. In Asia-pacific countries the infections caused by Gram negative bacteria is more prevalent that those caused by the Gram-positive bacteria in hospital admitted patients [4, 17]. For example, high mortality is associated with infections caused by multi-drug resistant *Ps.aeruginosa* [6, 8, 14]. Prevention and management of such bacterial infections in these patients is a challenge. Clinicians can manage the bacterial infections in better way if they know their drug resistance patterns and their prevalence in their regions.

The common bacteria which cause infections in co-morbid patients are *Esch.coli, Kleb. pneumoniae* [9, 11, 13] and non-fermentors such as *Acinetobacter spp.,* and *Pseudomonas* spp., and methicillin resistant *Staph.aureus*.

The indiscriminate use of β-lactam antibiotics exerts the selective pressure on the many Gram-negative and Gram positive pathogenic bacteria leading to the emergence of EMBL and MBL strains all over the world [18, 19].

The extended spectrum-β-lactamase and Amp C producing members of *Enterobacteriaceae* family and Metalo-β-lactamase(MBL) producing organisms including New Delhi MBL-1(NDM-1) strain are responsible for extended the hospital stay and contributing to increase morbidity and mortality among these patients[10].

Therefore, the continue surveillance of the prevalence of these bacterial pathogens with drug resistance help clinicians to prevent and manage such infections in better ways.

MATERIALS AND METHODS:

The total 214 clinical samples received from different clinical wards were subjected to microbial analysis during the period of October 2019 to April 2020 in a Tertiary Care Hospital. The 214 samples produced total 142 cultures of Gram positive and Gram negative bacterial pathogens. The isolated Gram positive and Gram negative bacteria were identified to the species level on the basis of phenotypic and biochemical tests and were subjected to antimicrobial susceptibility testing using CLSI guidelines of 2016[1]. The incidence of Gram negative bacteria in all the processed clinical specimens were tabulated and incidence of drug resistance of selected drugs was calculated for each antimicrobial agent.

The following antibiotics were chosen for Gram negative bacteria at their appropriate concentrations: Amoxicillin+clavulanate (20 μg /10 μg), Ampicillin+salbactam (10 μg /10 μg), Amikacin (30 μg), Ciprofloxacin (5 μg), Levofloxacin (5 μg), Imipenem (10 μg), and Meropenem (10 μg).

For Gram positive bacterial pathogens following antimicrobial agents were selected at their appropriate concentrations:

Gentamicin(30μg), Ciprofloxacin(5μg), Levofloxacin(5μg), erythromycin(15μg), Linezolid(30μg), Clindamycin(2μg), Vancomycin(30μg), Cefuroxime(30μg), Ceftizoxime(30μg), Ceftriaxone(30μg), Azithromycin(15μg), Cefoxitin(30μg), Ampicillin+sulbactam(10 μg /10μg), Amoxicillin+ clavulanic acid (20μg/10μg), Trimethoprim+ sulphame thoxazole (23.75μg/1.25μg).

RESULTS:

The total 214 clinical samples produced 106 and 36 cultures of Gram negative and Gram positive bacterial cultures respectively as shown in table No.1 & 2.

Table -1: Distribution of Gram negative bacterial isolates in different clinical samples

(N=142).

Sr. No	Clinical samples	*Esch. coli*	*Ps.aeruginosa*	*Klebsiella spp*	*Acinnetobacter*	*Proteus spp*
1	Urine	40	3	7	0	3
2	Sputum	0	0	3	0	0
3	Aspirates	0	4	4	10	0
4	Pus	7	11	1	2	0
5	Stool	1	0	1	0	0
6	Vaginal swab	4	0	3	0	0
7	Drain fluids	1	0	0	0	0

8	Tips	0	1	0	0	0
9	Total	53(37.32%)	19(13.38%)	19(13.38%)	12(8.45%)	3(2.11%)

Table -2: Distribution of Gram positive bacterial isolates in different clinical samples

(N=142)

Sr. No	Clinical specimens	Coagulase(+ve) Staphylococci *(Staph.aureus)*	Coagulase (-ve) *Staphylococci*	*Entero. faecalis*
1	Urine	0	2	3
2	Sputum	0	0	0
3	Aspirates	0	1	0
4	Pus	10	11	0
5	Stool	0	1	0
6	Vaginal swabs	0	4	4
7	Drain fluids	0	0	0
8	Tips	0	0	0
9	Total	10(7.0%)	19(13.38%)	7(4.92%)

The results of antimicrobial susceptible tests for various isolates were recorded as shown in table No.3. & 4.

The incidence of infections caused by Gram negative bacterial pathogens were found to be 74.64% whereas those caused by Gram positive bacterial pathogens was found to be 25.36%.The incidence of isolates of Gram negative bacteria was highest for *Esch.coli* (37.32%) followed by *Klebsiella* (13.32%), *Ps. aeruginosa* (13.32%), *Acinetobacter* (8.45%) and *Proteus* (2.11%) among isolates of Gram positive and Gram negative bacteria.

The incidence of UTI and pus infections caused by Gram positive and Gram negative bacterial Pathogens were recorded as 40.84% and 30.28% respectively.

Table- 3. Antibiotic resistance pattern of Gram negative organisms isolated from different clinical samples.
Resistance percentage (%) of Gram negative organisms

Sr. No	Antimicrobial agent	*Esch. coli* (n=53)	*Ps.aeruginosa* (n=19)	*Klebsiella spp* (n=19)	*Acinetobacter spp* (n=12)	*Proteus spp.* (n=3)
1	Amikacin	18.51	15.78	36.84	41.66	66.66
2	Aztreonam	75.92	47.36	73.68	100	66.66
3	Imipenem	68.51	42.10	75	66.66	100
4	Meropenem	9.25%	26.31	52.63	58.33	66.66
5	Ciprofloxacin	81.48	31.57	78.94	58.33	66.66
6	Leofloxacin	79.62	73.68	63.15	41.66	66.66
7	Ceftizoxime	85.18	84.21	68.42	100	66.66
8	Cefuroxime	92.59	100	73.68	100	100
9	Ceftriaxone	83.33		73.68	83.33	100
10	Ampicillin/ sulbactam	51.85	84.21	78.94	41.66	66.66
11	Amoxicillin/ clavulinc acid	90.74	73.68	73.68	75	100
12	Piperacillin/ tazobactam	29.62	42.10	47.36	75	33.33
13	Ceftazidime/ clavulanic acid	42.59	57.89	63.15	91.66	66.66
14	Trimethoprim/ sulphamethoxazole	68.51	84.21	52.63	66.66	100
15	Cefoperazone /Sulbactum	59.25	73.68	63.15	66.66	66.66

The incidence of drug resistance among the Gram positive bacterial pathogens were found to be 100% for more than two anti-microbial, same is true for the Gram negative bacterial pathogens.

Table -4: Antibiotic resistance pattern of Gram positive organisms isolated from different clinical samples.Resistance percentage (%) of Gram positive organisms.

Sr. No	Antimicrobial agent	Coagulase Positive Staphylococci (Staph.aureus) (n=10)	Coagulase Negative Staphylococci (n=19)	Enterococcus Faecalis (n=7)
1	Azithromycin	**70**	**89.47**	**100**
2	Cefuroxime	**70**	**42.10**	**100**
3	Ceftriaxone	**80**	**47.36**	**100**
4	Ceftizoxime	**70**	**42.10**	**100**
5	Cefoxitin	**70**	**26.31**	**0**
6	Ciprofloxacin	**60**	**36.48**	**85.71**
7	Clindamycin	**30**	**26.31**	**100**
8	Erythromycin	**60**	**47.36**	**100**
9	Gentamicin	**0**	**0**	**14.28**
10	Levofloxacin	**60**	**42.10**	**85.71**
11	Linezolid	**0**	**0**	**0**
12	Vancomycin	**0**	**0**	**0**
13	Trimethoprim/sulphameoxazole	**60**	**47.36**	**57.14**
14	Amoxicillin/clavulanic acid	**80**	**73.68**	**85.71**
15	Ampicillin/sulbactam	**80**	**21**	**85.71**

DISCUSSION:

All the isolates of Gram positive as well as Gram negative bacterial pathogens were found to be multi-drug resistant and the incidence of drug resistance among Gram negative bacterial pathogens was comparatively higher than the incidence of drug resistance among Gram positive bacterial pathogens. The all Gram negative bacteria have shown remarkable drug resistance to first generation beta-lactam antibiotics such as ampicilin, amoxicillin and second generation cephalosporins such as cefuroxime and third generation cephalosporins such as ceftizoxime and ceftriaxone. The isolates of *Esch.coli*, *Klebsiella, Ps.aeruginosa* and *Proteus* have exhibited 92.59%, 73.68%, 100%, 100% drug resistance respectively to cefuroxime antibiotic. The amoxicilline+clavulanic acid, ampicillin+sulbactam and piperacillin+taxobactam combinations are also showing high drug resistance among the isolated Gram negative bacteria; The amoxycillin+clavulanic acid combination is showing high drug resistance whereas the piperacillin+tazobactam is showing less drug resistance among all the Gram negative bacterial isolates. Comparatively, resistance to carbapenems such as imipenem and carbapenem show less incidence by these Gram negative bacteria. High drug resistance to second and third generations cephalosporins exhibited by these bacteria is matter of concern for the health professionals. The high incidence of drug resistance to first generation beta-lactam antibiotics and second and third generation cepahlosporins may be because of frequently or indiscriminate use of these antibiotics. Therefore, there should be regular stewardship and surveillance programs to check the trends of drug resistance.

In the present study we found that the meropenem was comparatively effective against *Esch.coli* with drug susceptibility as 90.75% and *Ps. aeruginosa* with drug susceptibility as 73.69%. Whereas the drug was found to be less effective against *Klebsiella, Proteus*. and *Acinetobacter spp.*. In the similar study conducted by Joshi et. al. [11] in 2019, meropenem was reported as more effective against the *Esch.coli* and *Ps.aeruginosa*.

The piperacillin+tazobactam combination was also found, to some extent, effective against the *Esch.coli, Ps.aeruginosa, Klebsiella* and *Proteus spp.* after meropenem whereas the drug combination was very less effective against the *Acinetobacter spp.*. The average susceptibility to piperacillin+tazobactam among all the Gram-negative bacterial isolates was found to be 54.52% contrarary to the 30 to 35% susceptibility of this combination in India in 2012[16]. This prove that the drug holiday for longer time span can reduce the drug resistance among the pathogenic bacteria.

Coagulase negative *Staphylococcus* showed higher drug resistance to azithromycin and amoxycillin+clavulanic acid as 89.47% and 73.68% respectively. Coagulase positive *Staph. aereus* show higher drug resistance to amoxycillin+clavulanic acid, ampicillin and ceftriaxone. The overall drug resistance to number of antibiotics is more in coagulase positive *Staph.aereus* than in coagulase negative *Staphylococcus*.

We found that among the three Gram positive bacterial isolates, the isolates of *Enterococcus faecalis* showed the higher drug resistance to almost all the antimicrobials tested. The drug resistance incidence for ceftraixone, ceftizoxime, cefuroxime and azithromycin is 100%, whereas the drug resistance to both ciprofloxacin and levofloxacin were found to 85.71%. Interestingly *Enterococcus faecalis* isolates showed no drug resistance to vancomycin and linezolid. In a similar

studies done by Shivali Khullar and et. al. in 2016[12], the incidence of drug resistance were reported highest in coagulase positive *Staph.aureus* followed by the *Enterococcus faecalis* among all the Gram positive bacterial pathogens. In our studies we found higher drug resistance incidence in *Enterococcus faecalis* followed by *Staph.aereus*.

CONCLUSION:

The incidence of infections caused by Gram negative bacterial pathogens was found to be higher than that of infections caused by the Gram positive bacterial pathogens. All the bacterial isolates were found to 100% multi-drug resistant. Majority of these isolated were showing higher resistance to the second and third generation penicillin and cephalosporins. Since the high incidence of drug resistance is associated with higher incidence of different types of bacterial infections [7], high morbidity and mortality, there should be constant drug resistance surveillance and strict implementation of stewardship program in the hospitals to minimise the emergence of drug resistance among pathogenic bacteria.

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