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Identification and Antibiogram of Gram Negative Bacterial Isolates from Pyogenic Samples in a Tertiary Care Hospital in Ajmer, Rajasthan

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Abstract

Background: Pus is an important clinical specimen received in Microbiology laboratory for culture and sensitivity. In pyogenic infection gram negative bacterial isolates are an important cause of sepsis. These infections are difficult to treat because of increasing antibiotic resistance in the pathogens.

AIM: To screen various gram negative bacterial isolates in the pus samples and determine their antibiotic sensitivity and resistance pattern against standard antibiotics.

Materials and Methods: It was an observational prospective study. A total of 352 pus samples received from various wards of JLN medical college & associated group of hospitals, Ajmer from Dec.2018 to June 2019 and were processed in the laboratory using standard microbiological procedures. Identification of isolates by using gram staining and biochemical tests .The antibiotic sensitivity testing of isolates were performed by Kirby- Bauer disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines.

Result: Out of 352 pus samples gram negative bacterial isolates were present in 180(51.13%) samples with Surgical wards (70%) being the major contributor. K. pneumonaie (30.05%) was the most common organism followed by P. aeruginosa (29.50%), E. coli 39(21.32%), E. aerogenes 29(15.85%), P. mirabilis 6(3.28%). Gram negative bacilli were susceptible to Imipenem (93.20%) and Aztreonam (53.80%). In Gram negative bacteria 30.60% were ESBL producers and 8.74% were MBL producer.

Conclusion: This study will guide the clinician in choosing appropriate antibiotics for better treatment and preventing emergence of the antibiotic resistance.

Keywords: Pus, ESBL, MBL

Introduction

Skin acts as a barrier and prevents the entry of microbes into our body unless the mechanism is breached due to any injury, trauma or surgical intervention. Thus wounds formed get infected by bacterial pathogens[1]. Pyogenic infection is characterized by several local inflammations, usually with pus formation, generally caused by one of the

pyogenic bacteria, which can produce the accumulation of dead leukocytes and infectious agents commonly known as pus[2]

In 1964, the National Academy of Sciences and the National Research Council USA published a classification scheme for surgical wounds that has been widely accepted which includes clean, clean contaminated, contaminated and dirty wounds[3]. The emergence of drug-resistant organisms in both hospitals and the community is a major concern. Surveillance studies have provided important information about changes in the spectrum of microbial pathogens and trends in the antimicrobial resistance patterns in nosocomial and community acquired infections and continued monitoring of antimicrobial resistance patterns in hospitals is essential to guide effective empirical therapy.

Focusing on this ever increasing issue of multidrug resistance due to inappropriate use of antibiotics, the current study was undertaken in our department to provide adequate and relevant data for proper selection and use of antibiotics.

Material And Method

This prospective study was conducted at Microbiology department, JLN Medical College, Ajmer. The study was conducted from December 2018 to June 2019. The study was approved by Institutional Ethical Committee.

Acceptance criteria: Pus samples were collected aseptically by sterile aspiration in sterile containers or sterile swab in sterile test tube and were accepted for this study.

Rejection criteria: Pus samples which were received in unsterile containers, dried pus, or sample without appropriate label**.**

Pus samples were collected with sterile disposable cotton swabs and aspirates in syringe and were transported and processed in the microbiology laboratory immediately. They were inoculated on to Blood agar (BA), MacConkey agar (MA) and Nutrient agar (NA). Culture plates were incubated at 37°C for 24 hrs to 48 hrs in aerobic condition. After incubation, identification of bacterium from positive cultures was done with a standard microbiological technique which includes motility testing by hanging drop preparation, gram staining and biochemical reactions such as catalase, indole, methyl red, citrate, urease, triple sugar iron test, phenyl pyruvic acid test and oxidase test. The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines and classified as sensitive, intermediate and resistant. Amikacin(30µg), Ampicillin(10mg), Aztreonam(50µg), Cefepime(30µg), Cefuroxime(30µg), Ceftazidime(30µg) Ciprofloxacin(5µg), Imipenem(10mg), Gentamicin(10 mg), Tobramycin(10 mg), Ceftazidime- clavulinic acid(30/10 µg).

Phenotypic Detection of ESBL Positive Isolates

Isolates resistant to Ceftazidime were tested for ESBL production by double disc diffusion test. A disc of ceftazidime(30µg) and ceftazidime/clavulanic acid (30/10µg) were placed on Mueller Hinton agar plate, and was incubated overnight at 37°C. An increase of ≥5 mm in the zone of inhibition in a disc containing clavulanic acid compared to the drug alone was considered as positive for ESBL producers.

Phenotypic Detection of MBL Positive Isolates

Isolates resistant to imipenem and/or meropenem were tested for MBL production by double disc diffusion test. A disc of imipenem (10µg) and imipenem + EDTA (10µg/750µg) were placed on Mueller Hinton agar plate, and was incubated overnight at 37°C. A

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zone difference greater than or equal to 7 mm around imipenem and imipenem + EDTA disc was interpreted as MBL positive isolate.

Results

Out of 352 samples gram negative bacilli were present in 180(51.13%) samples with Surgical wards (70%) being the major contributor.

Table 1: Wards wise distribution of pus samples

Name of wards	No. of Samples	Percentage
SURG. W	126	70%
BURN W	34	18.89%
ORTHO.W	5	2.78%
MED.W	4	2.22%
ENT W	2	1.11%
CH. W	1	O.56%
ICU	8	4.44%
Total	180	100%

Table 2:Various isolates obtained from pus samples

S. no.	Organisms	No. of organisms	Percentage (%)
1.	Klebsiella pneumoniae	55	30.05%
2.	Pseudomonas aeruginosa	54	29.50%
3.	E. coli	39	21.32%
4.	Enterobacter aerogenes	29	15.85%
5.	Proteus mirabilis	6	3.28%
	Total	183	100%

Table 3: Antibiogram of GNB (Enterobacteriaceae)

Organism		K.pneumoniae	E. coli	E. aerogenes	P. mirabilis
AK	S	47 %	77%	34%	50%
	R	53%	23%	66%	50%
AMP	S	09%	15%	07%	67%
	R	91%	85%	93%	33%
AMC	S	15%	33%	17%	33%
	R	85%	67%	83%	67%
AT	S	27%	64%	34%	83%
	R	73%	36%	66%	17%
CPM	S	35%	41%	24%	67%
	R	65%	59%	76%	33%
CXM	S	09%	13%	07%	17%
	R	91%	87%	93%	83%
CAZ	S	13%	31%	10%	33%
	R	87%	69%	90%	67%
CAC	S	53%	51%	55%	67%
	R	47%	49%	45%	33%
CIP	S	15%	23%	14%	00%
	R	85%	77%	86%	100%
IMP	S	84%	100%	97%	100%
	R	16%	0%	03%	00%
TOB	S	27%	64%	28%	50%
	R	73%	36%	72%	50%

Table 4: Antibiogram of P. Aeruginosa (n=54)

AK	S	54 %
	R	46 %
AT	S	61%
	R	39%
CPM	S	56%
	R	44%
CAZ	S	39%
	R	61%
CAC	S	63%
	R	37%
CIP	S	43%
	R	57%
IMP	S	85%
	R	15%
PIT	S	87%
	R	13%
TOB	S	48%
	R	52%

Table 5: ESBL distribution

Bacterial isolates	Total No.	No. of ESBL	ESBL %
K. Pneumoniae	55	22	40%
P. aeruginosa	54	12	22.22%
E. coli	39	8	20.51%
E. aerogenes	29	12	41.37%
P. mirabilis	6	2	33.33%
TOTAL	183	56	30.60%

Table 6: MBL distribution

Bacterial isolates	Total No.	No. of MBL	MBL %
K. Pneumoniae	55	9	16.36%
P. aeruginosa	54	7	12.96%
E. coli	39	0	00%
E. aerogenes	29	0	00%
P. mirabilis	6	0	00%
TOTAL	183	16	8.74%

Out of 183 Gram-negative isolates, 56 were ESBL producers and 16 MBL producers. K. Pneumoniae

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showed maximum ESBL production (40%) and MBL production (16.36%).

Discussion

In our study K. pneumoniae was the predominant organism 55 (30.05%) followed by P. aeruginosa 54 (29.50%). Our study is comparable with Vijeta et al (2015), Kritu Panta et al (2013) and Dr. R. Sarathbabu et al, (2012) have found Klebsiella spp. as the predominant organism present in wound infection. K. pneumoniae isolates were found to be 84% susceptible to imipenem similar to observations made by Dr. Naomi K (2012) 88%. While 100% Susceptibility observed by Sukumar et al (2017). Most of P.aeruginosa isolates were found sensitive to pipra-tazobactam (87%), imipenem (85%) and resistant to ceftazidime and comparable with the study of Dr. Naomi K (2012) and Su kumar et al (2017).

In our study ESBL and MBL was found in 30.60% and 8.74% respectively. Mita D.W. et al, (2013) observed 43% and 18% respectively and Sudhaharan et al, (2018) observed ESBL (32.90%).

Conclusion

The early detection of beta lactamase producing isolates would be important for the reduction of mortality rates for patients and also to avoid the intra hospital dissemination of such strains. To overcome the problem of emergence and the spread of multidrug resistant organisms, a combined interaction and cooperation between the microbiologists, clinicians and the infection control team is needed.

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