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Emerging trend of ESBL and MBL producers in exudate samples, A study from a tertiary care center of Wayanad

Linda Rose Jose[1], Champa H[2]
[1](Department of Microbiology, Dr.Moopen's Medical College,India)
[2](Department of Microbiology, Dr.Moopen's Medical College, India)

Abstract:
Background*: The introduction of antimicrobials have influenced the modern medical care in a massive way. Antimicrobial resistance (AMR) is a serious threat to the modern world. Enterobacteriaceae, a family encompassing many clinically important bacterial species, exhibits rising levels of AMR. Infection with either extended spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) or carbapenemase-producing Enterobacteriaceae (CPE) is associated with increased mortality rates, length of stay in the hospital and overall healthcare costs.*
Materials and Methods:*The study was conducted in Department of Microbiology, Dr. Moopen's Medical College, Wayanad during a period of six months. The isolates were identified on the basis of conventional microbiological procedures.* ***Results****: A total of 231 isolates were obtained from142 patients. The magnitude of ESBL producing GNB was 38.2 % and that of MBL was 6.5%. Out of the total number of Staphylococcus aureus isolated, the magnitude of MRSA was 50% .*
Conclusion: *The study concludes the increasing trend of ESBL and MBL producers in exudate samples along with MRSA and the need for effective measures to reduce morbidity and mortality among patients due to this menace.*
Key Words: *Antimicrobial resistance, ESBL, MBL, MRSA, exudate samples*

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I. Introduction

Modern medical care has been influenced to a greater extend by the development and introduction of antimicrobial agents. In the healthcare sector ,inappropriate prescribing and use of antibiotics have been regarded as one of the major causes of patient deaths resulting from AMR and are projected to reach 10 million annually by 2050. AMR will also cause losses in the trillions to global economic output [1]. ESBL-E and CPE have spread globally[2,3] and technologies such as whole-genome sequencing (WGS) are providing detailed insights into their evolution and dissemination which reveals the abuse and misuse of antibiotics. The infecting pathogens vary according to the place of occurrence and the environment of growth [3]. An exudate is any fluid that filters from the circulatory system into lesions or areas of inflammation. It can be a pus like or clear fluid. In this study we have taken into account various exudate samples including pus, wound swab, drain tip, umbilical swab, vaginal swab, bed sore, abscess, ascitic fluid, catheter tip, peritoneal fluid, pericardial fluid and ear swab. Establishment of an appropriate and rational antibiotic policy is therefore essential to control this growing danger. ESBLs and MBLs producing organisms are a serious threat today along with the MRSA isolates. This is creating a very serious therapeutic difficulty which in turn affects the outcome for the patients. The increased use of antibiotics, especially during the pandemic has resulted in wide spread antibiotic resistance especially among the Gram negative bacteria. Earlier the incidence of antibiotic resistant organisms were in immunosuppressed individuals. There are several studies depicting the occurrence of drug resistant pathogens in healthy individuals. This study is aimed at analyzing the antibiotic susceptibility profile of pathogens isolated from exudate and detection of ESBLs and MBLs.

II. Material And Methods

The study was conducted in Department of Microbiology, Dr.Moopen's Medical College, Wayanad. Samples were obtained between December 2020 and May 2021. The isolates were identified on the basis of conventional microbiological procedures .The identified isolates were subjected to Antimicrobial susceptibility Testing and antibiotic susceptibility of the isolates was determined by Kirby Bauer Disk Diffusion method. The results were recorded and interpreted according to the standard guidelines (CLSI). This was a laboratory based

study. The specimen sources and patient information such as sex, age and setting, were carefully recorded from laboratory request forms.

Procedure methodology

Detection of ESBL Phenotypic confirmatory test

Test organisms were inoculated into Mueller-Hinton agar as lawn culture. The isolates resistant to clavulanic acid was subjected to the ESBL phenotypic confirmatory test. The ceftazidime (30 g) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 g discs) were placed. An increase of 5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer[4].

Detection of MBL: Imipenem EDTA combined disc test

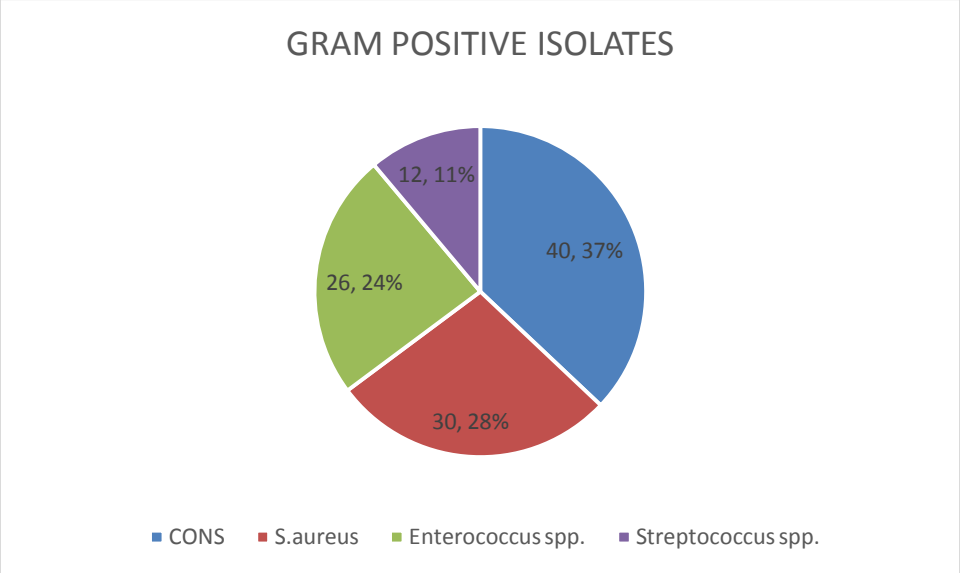
Test organisms were inoculated into Mueller-Hinton agar as lawn culture. The isolates resistant to Imipenem was subjected to the MBL detection test .One (10 mcg) imipenem disc and Imipenem–EDTA (10-750 mcg) disc was placed on a plate inoculated with the test organism and was used for screening of Metallo-β-lactamases producers. A zone diameter difference of ≥ 7 mm between Imipenem discs & Imipenem plus EDTA discs were interpreted as Metallo-β-Lactamase positive[4].

III. Results

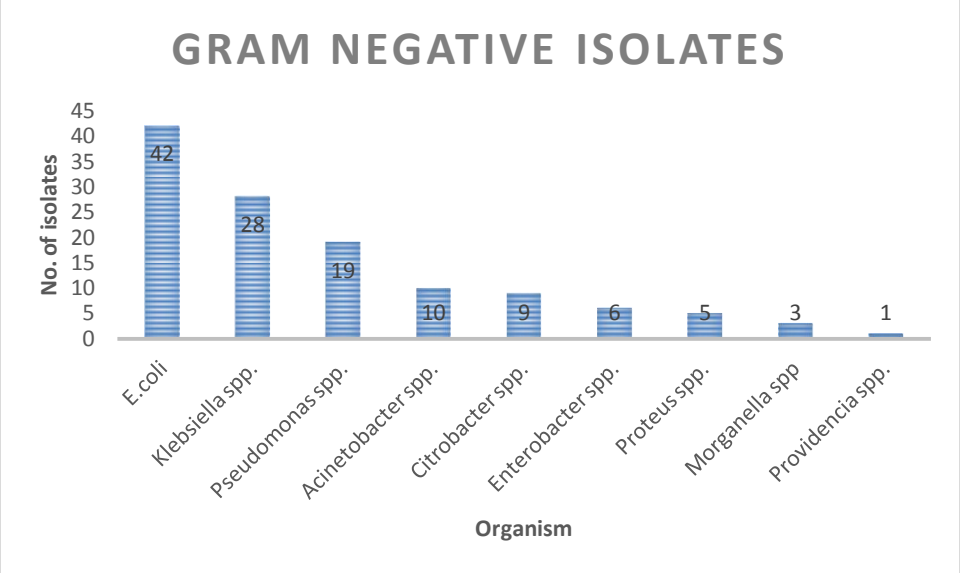
A total of 231 clinical isolates were obtained from different exudate samples which included pus, wound swab, drain tip, umbilical swab, vaginal swab, bed sore, abscess, ascitic fluid, catheter tip, peritoneal fluid, pericardial fluid and ear swab from 142 patients. Table:1shows the isolates from different samples. This included 108 (46.8%) Gram positive and 123 (53.2%) Gram negative bacteria. Among the 142 patients 85 (59.9%) were males and 57(40.1%) were females and in this 40.3% of the isolates were obtained from females and 59.7% were from males. Among the Gram positive isolates CONS 40(37%) were the predominant isolate followed by *S.aureus* 30(28%), *Enterococcus spp*. 26(24%) and *Streptococcus spp.* 12(11%). *E.coli* 42(34.13%) was the predominant pathogen among the Gram negative isolates followed by *Klebsiella spp*. 28(22.8%),*Pseudomonas spp.*19(15.5%), *Acinetobacter spp.*10(8.1%),*Citrobacter spp*.9(7.3%), *Enterobacter spp*.6(4.9%), *Proteus spp.* 5(4.07%), *Morganella spp.* 3(2.4%), *Providencia spp.* 1(0.8%). Graph:1 shows the occurrence of Gram positive isolates and Graph:2 shows the incidence of Gram negative isolates. Table No.2 and Table No.3 shows the antimicrobial susceptibility pattern of Gram positive and Gram Negative isolates. Table:4 shows the antimicrobial resistance pattern of ESBL and MBL producers with *E.coli* (59.5%)being the predominant isolate with ESBL producing ability followed by *Klebsiella* spp.(21.2%), *Citrobacter spp*.(6.4%), *Acinetobacter spp*. and *Enterobacter spp*(4.3%) and *Pseudomonas spp.* and *Proteus spp.* (2.1%). The MBL producers included *E.coli*(62.5%) and *Klebsiella spp*. (37.5%).

Table no 1 : Shows the different pathogens isolated from exudate samples

Organism	No.of isolates	Pus	Wound swab	Drain tip	Umbilical swab	Vaginal swab	Bed sore	Abscess	Ascitic fluid	Catheter tip	Peritoneal fluid	Pericardial fluid	Ear swab
E.coli	42	34	1	1				1	4	1			
Klebsiella spp.	28	23		1		1	1		2				
Pseudomonas spp.	19	11		1						1	1	1	4
Acinetobacter spp.	10	7		1	1	1							
Citrobacter spp.	9	7					1						1
Proteus spp.	5	5											
Enterobacter spp.	6	6											
Morganella spp.	3	3											
Providencia spp.	1	1											
Total	123	97	1	4	1	2	2	1	6	2	1	1	5



Graph1: Gram positive organisms isolated in the study



Graph:2 Gram negative organisms isolated in the study

Table no 2: Records the antimicrobial susceptibility pattern of Gram negative isolates

Organisms (No.of isolates)	AMP (%)	AMC (%)	G (%)	AK (%)	CIP (%)	CAZ (%)	CTX (%)	IMP (%)	MER (%)	PIT (%)	CFS (%)	COT (%)
E.coli (n=42)	4.8	28.6	70.7	90.2	19	28.6	32.5	100	100	82.9	65.9	47.61
Klebsiella spp.(n=28)	3.6	33.3	62.5	83.3	34.8	29.2	41.7	80	80	65.2	58.3	50
Pseudomonas spp.(n=19)	29.8	65	69.2	100	66.7	38.5	100	100	100	83.3	76.9	52.3
Acinetobacter spp.(n=10)	72.1	75	70	56	70	74.6	74	30.1	30.1	44.4	56	28.2
Citrobacter spp.(n=9)	50.1	57.1	100	100	100	100	100	100	100	100	100	100
Enterobacter spp.(n=6)	50	50	68.5	83.3	50	33.3	50	100	100	83.3	83.3	83.3
Proteus spp.(n=5)	80.2	100	100	50	100	100	100	100	100	100	100	100
Morganella spp(n=3)	100	100	100	50	100	100	100	100	100	100	100	100
Providencia spp.(n=1)	100	100	100	100	100	100	100	100	100	100	100	100

AMP – Ampicillin, AMC – Amoxyclav, G – Gentamycin, AK – Amikacin, CIP – Ciprofloxacin, CAZ – Ceftazidime, CTX – Cefotaxime, IMP-Imipenem, MRP –Meropenem, PIT – Piperacillin-Tazobactum, CFS-Cefoperazone sulbactam, COT-Cotrimoxazole

Table no3: Shows the antimicrobial susceptibility pattern of Gram positive isolates

Organisms (No.of isolates)	AMP (%)	AMC (%)	G (%)	AK(%)	CIP (%)	CLI(%)	RIF(%)	E (%)	LZ(%)	PRI (%)	DOX (%)	COT (%)	VAN (%)	TGC (%)
S.aureus (n=30) MRSA (n=15)	90	28.6	90	90	33.3	70	90	32	100	56.7	100	60	70	100
CONS (n=40)	27.5	27.5	60	83.3	60.5	62.5	94.9	17.5	100	25	87.5	43.6	97.5	100
Streptococcus spp.(n=12)	100	100	58.3	100	100	60	100	58.3	100	75	76.9	58.3	75	83.3
Enterococcus(n=26)	76.9	-	70.8	-	100	50	-	-	88.5	15.4	-	-	100	-

AMP – Ampicillin, AMC – Amoxyclav, G – Gentamycin, AK – Amikacin, CIP – Ciprofloxacin, CLI-Clindamycin, RIF-Rifampicin,E-Erythromycin, LZ-Linezolid, PRI-Pristinamycin, DOX- Doxycycline, COT-Cotrimoxazole, VAN- Vancomycin, TGC-Tigecycline

Table: 4 ESBL and MBL mediated resistance in organism

No. of Gram Negative isolates	No. of ESBL producers	No. of MBL producers
123	47 (38.2%)	8 (6.5%)

IV. Discussion

Wound infections are a serious threat to the patients as well as health care system[5]. Currently there is a limitation of data available in this regard. This study is carried out to identify the pathogens from various exudate sample and to create awareness against the overuse of antibiotics. Most of the times the infections can be monomicrobial or polymicrobial. The predominant pathogen included *E.coli* 42(34.13%) and the least was *Providencia spp.* 1(0.8%) E.coli. Among the Gram positive isolates CONS 40(37%) were the predominant isolate followed by *S.aureus* 30(28%), *Enterococcus spp*. 26(24%) and *Streptococcus spp.* 12(11%). The occurrence of E.coli as the predominant pathogen is similar to many other studies. There are studies which discussed about ESBL and MBL producers where *Pseudomonas spp*. and CONS were the predominant pathogens isolated. The present study is in discordance with the studies done by Swati Duggal et.al[6]. where E.coli was not the predominant Gram negative pathogen. This study is not in concordance with the studies done by Kumari PH et al[7]. and Wadekar et.al.[8] where *S.aureus* were the predominant pathogen among Gram positive isolates, but in the present study CONS were found to be the predominant pathogen.

The resistance to ß-lactam group of antibiotics are shown by a wide range of Gram Negative organisms. In this study 38.2% of Gram Negative organisms were ESBL producers which is similar to the studies done by Ghotaslou R *et.al.*[9] and in discordance with the study by Jose LR.et.al[10] .Methicillin-resistant Staphylococcus aureus (MRSA) strains are resistant to a large group of antibiotics called beta-lactams. Methicillin resistance is caused by the acquisition of a mecA gene. This produces an alternative penicillin- binding protein 2a (PBP2a), which has lower affinity for b -lactam antibiotics. This study is in concordance with the studies of Wadekar et.al.[8] Drug resistance among bacteria is a serious threat to the world which should be dealt with in a timely manner and with the appropriate strategies.

V. Conclusion

To combat resistance irrational use of antibiotics should be avoided. Introduction of continuous surveillance programs, molecular characterization of carbapenemase producers and regular publication of antimicrobial susceptibility patterns can prove to be effective tools to control drug resistance. Better surveillance guidelines when implemented appropriately will help in reducing morbidity and mortality.

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