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Prevalence of MRSA and Beta-Lactamase-Producing Bacteria in Pus Samples

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KEYWORDS	Abstract: Background:	Swift dissemination of b	acteria in wound infections	and the timely
s.aureus,susceptibility	administration of appropri	riate antibiotics are of utmos	st importance for healthcare pr	ofessionals. The
,lactamase.	aim of this current resear	ch was to assess the prevaler	nce and spatial distribution of b	oacteria obtained
	from purulent samples,	as well as to examine the	neir antibiotic susceptibility	patterns among
	hospitalized patients. Me	thods: A cross-sectional inv	vestigation was conducted ove	r a span of one
	year. During this period,	samples of pus were obtain	ned from the patients. Bacteri	al isolation and
	identification were accom	plished through culture and l	piochemical tests, while antibio	tic susceptibility
	testing was performed us	sing the disc diffusion meth	od. Results: S. aureus emerg	ged as the most
	frequently isolated bacter	eria. Gram-positive bacteria	a displayed notable resistance	e to a range of
	antibiotics, including Per	nicillin, Cefoxitin, Ampicilli	n, Azithromycin, Cotrimoxazo	ole, Cefuroxime,
	and Cepradine.Remarkabl	ly, 58.1% of S. aureus and 10	0% of S. epidermidis demonstra	ated resistance to
	Methicillin, while 51.2%	of the isolates were identified	ed as AMPC β -Lactamase pro-	ducing bacteria.
	Conclusion: The escalation	ng misuse of antibiotics has l	led to a surge in Methicillin-res	istant, AMPC β -
	Lactamase, and ESBL-pr	oducing bacteria. Our resea	rch underscores a concerning	trend of rapidly
	increasing multidrug-resis	tant (MDR) bacteria, which p	presents a significant challenge.	Consequently, it
	is imperative to conduct a	ntibiotic susceptibility testing	g prior to antibiotic administrat	ion, and there is
	an urgent need for ongo	oing monitoring of antibiot	ic sensitivity to mitigate the	development of
	resistance.			

I. INTRODUCTION

Wound infections are a prevalent source of morbidity, often leading to extended hospital stays. Consequently, effective infection control plays a pivotal and intricate role in wound care, particularly in the face of the global surge in antimi- crobial resistance, which poses a significant challenge. Mi- crobial pathogens play a significant role in the development of human skin and soft tissue infections (SSTIs), which often occur during or after trauma, burn injuries, or surgical procedures. These infections result in the formation of pus, a whitish to yellowish fluid comprising dead white blood cells (WBCs), cellular debris, and necrotic tissues. Among the prominent pus-producing bacteria are Staphylococcus aureus (S. aureus), Klebsiella pneumoniae, Pseudomonas, Escherichia coli, and Streptococci, with S. aureus being the most prevalent offender. These bacteria are frequently associated with wound infections and are widely distributed within healthcare settings, causing substantial morbidity and imposing a significant financial burden on healthcare sys- tems. To investigate their virulence and antibiotic suscep-tibility, it is imperative to obtain a pure bacterial culture. Antibiotic resistance arises due to the improper use of med- ications, and it rapidly spreads among highly concerning bacterial strains, posing significant threats to public health on a global scale. Recent research has suggested that combining different antibiotics might offer an effective approach to address this challenge. The emergence and proliferation of antibiotic-resistant bacteria represent a grave and imminent threat to human well-being. The diminishing number of new antibiotics



in development amplifies the alarm surrounding the multidrug-resistant, emergence of pathogenic organisms, posing grave threats to both individual patients and public health at large. It is worth noting that ESBL-producing strains may be more prevalent than acknowledged, often eluding currently routine screening methods. These ESBL strains are also linked to resistance against non- β -lactam antibiotics, such as aminoglycosides and Chloramphenicol. Continu-ously monitoring bacterial isolates and identifying antibiotic resistance patterns is a pressing concern for microbiologists. Furthermore, doctors are placing a significant emphasis on addressing critical issues like multidrug resistance, recogniz- ing the urgency of the situation.

II. MATERIAL AND METHODS

For this research, a Cross-Sectional Study design was chosen, and a purposive sampling technique was employed. The study was conducted within the Microbiology Laboratory and spanned a duration of one year. Data collection for this study involved the use of a questionnaire, through which information such as age and gender were gathered. A total of 100 pus samples were collected from the Microbiology Department. Following the collection of these samples from the laboratory, each sample was accurately labeled and promptly transported to the Microbiology laboratory while maintaining a temperature of 2-4°C to ensure sample integrity. The collected pus samples underwent a series of processing steps, which included Gramstaining and culturing. In an aseptic manner, these samples were inoculated onto Blood agar, Brain Heart Infusion agar, and MacConkey's agar plates, followed by incubation under aerobic conditions at a temperature range of 35°C to 37°C for a period of 24 to 48 hours. Subsequent identification and characterization of the isolates were carried out using well-established microbiological techniques in accordance with standard protocols. The antimicrobial susceptibility of all the selected isolates was assessed using a standardized double-disk diffusion method known asthe Kirby-Bauer method. Specifically, antibiotic susceptibility testing included screening for ESBL (Extended-SpectrumBeta-Lactamase) production, which was determined by observing specific zone diameters. A high level of suspicion for ESBL production was raised when testing with antibiotics like Cefuroxime, Ceftazidime, Aztreonam, Cefotaxime, or Ceftriaxone, as per

guidelines established by the Clinical and Laboratory Standards Institute (CLSI). The CLSI has developed disc diffusion protocols designed to screen for ESBL production in bacteria such as Klebsiella, E. coli, and Proteus spp. If any of the zone diameters from these tests indicate suspicion for ESBL production, additional confir- matory tests should be conducted to establish the diagnosis. Methods for detecting AmpC β -lactamaseproducing isolates are available, but they are continuously evolving and have not yet been fully optimized for routine use in clinical laborato- ries. This can lead to an underestimation of these resistance mechanisms. In some cases, carbapenemases can be used to treat infections caused by AmpC-producing bacteria, but it's important to note that carbapenem resistance can developin certain organisms due to mutations that either reduce the influx (loss of outer membrane porins) or increase the efflux (activation of efflux pumps) of carbapenem antibiotics.

III. RESULTS

Pus samples were obtained from a total of 100 patients, with 36 (36%) being indoor patients and 64 (64%) being outdoorpatients. Among these 100 patients, the majority fell within the age group of 40 to 50 years. In terms of gender distribu- tion, males were more predominant, comprising 56% (56%),

Gender	Frequency	Percentage
Male	56	56%
Female	34	34%
Total	100	100%

TABLE 1: Distribution of gender among study population (n=100)

Gender	>40 yrs	40-50 yrs	50-60yrs	>60yrs
Male	14(8%)	41(22%)	6(12%)	17(14%)
Female	8(16%)	5(10%)	4(8%)	5(10%)
Total	22(24%)	46(32%)	10(20%)	22(24%)

TABLE 2: Distribution of age and sex among study popula-tion (n=100)

while females accounted for 44% (44%). Furthermore, the culture yielded positive results in the majority of the samples, specifically in 76% (76%) of them. The prevalent bacteria identified were primarily S. aureus, followed by Klebsiella pneumoniae and E. coli. S. aureus exhibited high resistance to a range of antibiotics including Penicillin, Cefoxitin, Ampicillin,

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Cotrimoxazole. Cefuroxime. Azithromycin, and Cepradine, while showing lesser resistance to Amikacin and Gentamicin. Out of the 24 S. aureus isolates, 100% were identified as MRSA (Methicillin-Resistant Staphylococcus aureus), while a single S. epidermidis isolate (20 out of 20) was identified as MRSS (Methicillin-Resistant Staphylococ- cus epidermidis), and 4 (21.1%) were identified as VRSA (Vancomycin-Resistant Staphylococcus aureus). Klebsiella pneumoniae displayed 100% resistance to Cepradine and Cefuroxime, followed by high resistance to Cotrimoxazole (84.71%) and Cefixime (70.42%). Among the gram-negative bacilli, 22 (52.2%) were found to be AMPC β -Lactamase positive, 13 (13.0%) were ESBL positive, and 9 (8.7%) were positive for both AMPC β -Lactamase and Carbapenemase.

The antibiotic resistance pattern among gram-positive isolates, particularly S. aureus, revealed significant resis- tance levels. S. aureus displayed the highest resistance rates to Penicillin (100%) and followed closely by Ampicillin, Azithromycin, Cefuroxime, Cepradine, and Cotrimoxazole (all at 92.65%). Conversely, it exhibited the lowest resis- tance to Gentamicin, Amikacin, Cefixime, and Cefotaxime. However, S. aureus exhibited varying degrees of resistance to other antibiotics, with resistance rates for Ciprofloxacin at 85.71%, Clindamycin at 78.57%, Amoxiclav, Doxycycline, Cefoxitin at 57.14%, and Ceftazidime at 50%. On the other hand, a single S. epidermidis isolate displayed complete sensitivity (100%) to Amikacin and Gentamicin but was entirely resistant to all other tested antibiotics.

IV. DISCUSSION

Wound infections have emerged as the most prevalent hospital-acquired condition, and the hospital environment plays a crucial role in the occurrence of these infections, as observed in previous research (Khanam et al., 2018) .In our

current study, among the 100 participants, a higher number were male, followed by females. This gender distribution aligns with the findings of another study conducted by Batraet al. (2020), where males were also predominant, account- ing for 58.6% of the cases compared to females. In this study, the mean age of the participants was approximately 40 years, aligning with the findings of a study by Karmaker et al. in 2016. Out of the 100 pus samples collected, the majority were from the outpatient department (OPD) at 65%, followed by the inpatient department (IPD) at 35%. Additionally, a significant proportion (76%) of these samples showed positive growth, consistent with findings reported by Trojan et al. in 2016 . Regarding the bacterial composition, gram-positive bacteria were present in 38.5% of the samples, whereas gram-negative bacteria were present in 60.8%. This is comparable to the findings in a study by Rai et al. in2017, where 61% of growth positive samples were gram- positive and 39% were gram-negative. Specifically focusing on gram-positive cocci, out of the 15 isolates, S. aureus was the predominant bacterium, constituting 93.4% of the cases. Among these S. aureus isolates, 66.7% were from outdoor patients, and 26.7% were from indoor patients. This study highlights the prevalence of gram-negative bacteria, with a total of 23 isolates identified. Among these, Klebsiellapneumoniae was the most frequently encountered organism, comprising 30.4% of the cases, followed by E. coli at 17.3%, Proteus spp. at 13%, Enterobacter spp. at 13%, Acinetobacter at 23%, and Pseudomonas at 23%. These findings align with the results reported in previous studies. For instance, Wang et al. (2018) identified Klebsiella as the most common pathogen in liver abscess infections (80.3%), followed by E. coli (7.8%), Pseudomonas aeruginosa (1.8%), and Acinetobacter baumannii (1%). Similarly, Thanni et al. (2003) found that Pseudomonas spp. accounted for 27.9% of cases, followed by Klebsiella spp. at 16.5%, Proteus spp. at 15.1%, and E. coli at 7%. Serraino et al. (2018) also observed E. coli as the mostcommon pathogen at 26.5%, followed by Klebsiella spp. at 4.6%, Proteus spp. at 1.9%, and Citrobacter spp. at 1.9%. Furthermore, in this study, S. aureus exhibited high resistance rates to penicillin (100%) and also showed notable resistance to Ampicillin, Azithromycin, Cefuroxime, Cepradine, and Cotrimoxazole, with a resistance rate of 94.85

V. CONCLUSION

Staphylococcus aureus was the most frequently isolated gram-positive bacterium, while among gram-negative bacilli,Klebsiella pneumoniae and Escherichia coli were the pre- dominant bacteria responsible for wound infections in this study. The study also provides valuable insights into the an-tibiotic resistance patterns of these bacterial isolates. This in- formation can be instrumental in developing a local antibiotic policy for the hospital and initiating appropriate empirical antibiotic treatment before the availability of culture

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reports, thereby enhancing patient care and management.

AUTHORS CONTRIBUTION

All authors equally contributed in the writing of the paper.

CONFLICT OF INTERESTS

Author declared that there is no conflict of interest.

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