RESEARCH

BACTERIAL ETIOLOGY AND ANTIBIOGRAM OF SURGICAL WOUND INFECTION AT A TERTIARY CARE HOSPITAL, WESTERN INDIA

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Abstract

Background: Wound infections are caused due to microbial proliferation at the particular site following skin damage. Prompt microbiological identification and antimicrobial sensitivity testing leads to appropriate antibiotic selection and prevents escalation of antimicrobial resistance. The aim and objectives were to evaluate the bacteriological profile and antibiotic susceptibility pattern of wound infections. **Materials and Methods:** A retrospective analysis of microbiological laboratory records of all clinical samples received during the study period, from surgical ward, was done based on findings of primary smear, culture isolates and their antibiotic sensitivity. All laboratory procedures were performed, as per standard protocol. Antimicrobial susceptibility testing of the bacterial isolates by Kirby-Bauer disk diffusion method was performed and interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines. **Result:** The total samples studied were 180. 100/180 samples showed growth of organisms on culture. Total number of culture isolates were 116. Majority were Klebsiella (22%) followed by E.coli (19%), MRSA(17%), Pseudomonas(16%), Staphylococcus aureus (9%), Acinetobacter(6%) and others (eg Streptococcus, Morganella morganii, Proteus, Enterobacter,CONS, Citrobacter and Candida). In 16/100 samples more than one(mixed) organisms were found. Majority of the Gram-negative organisms (60%) were sensitive to amikacin, gentamicin, piperacillin-tazobactum and amoxicillin/clavulanate. Majority of the Gram-positive organisms (75%) were sensitive to vancomycin, linezolid, clindamycin, gentamicin, doxycycline and tetracycline. **Conclusion:** Definitive management of wound infections is most important in hospital setup, where patients present for immediate treatment. A predictable bacterial profile and antibiotic sensitivity in wound infections will be of great help for clinicians to start empirical treatment.

INTRODUCTION

Wound infection continues to be a challenging problem and represents a considerable healthcare burden.[1] The success of current therapies is fraught with adverse wound microenvironment, chronicity, and biofilm formation, thus impeding adequate concentrations of active antimicrobials at the site of infection. Its a therapeutic challenge as several complex factors and conditions have to be considered in wound assessment, management and the overall healing process.[2] In recent years, the incidence of infected wounds is steadily increasing, and so is the clinical as well as economic interest in effective therapies. These are directed towards reduction of pathogen load in the wound with general wound management to facilitate the healing process. Advanced drug delivery systems have the potential to enable the tailor-made application of antimicrobials to the site of action, resulting in an effective treatment with negligible side effects.[3]

Early recognition along with prompt, appropriate and effective intervention are important in reducing the economic and health consequences, especially in the context of growing resistance to antibiotics.[4] Indiscriminate use of antibiotics has contributed to the development of antibiotic-resistant strains of bacteria (e.g. methicillin resistant Staphylococcus aureus (MRSA), vancomycin resistant Staphylococcus aureus (VRSA) and multi-drug resistant Gram negative pathogens).[5] However when used appropriately, systemic antibiotics can be potentially lifesaving in the management. Empirical antibiotic treatment must take into account the local antimicrobial susceptibility patterns of the possible pathogens. Implementation of surveillance followed

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by feedback of appropriate data to clinicians is of strategic importance.[6,7]

Aims and Objectives of the Study

1. To isolate and identify bacterial pathogens associated with wound infection
2. To study antimicrobial susceptibility patterns of bacterial pathogens

MATERIALS AND METHODS

A retrospective study was conducted from Oct 2021 till May 2022, in the Microbiology department of a tertiary care hospital. All the data was collected in a period of two month from the laboratory records and from medical record department.

*Inclusion criteria***:** All wound samples received from surgical ward of our hospital during the study period.

*Exclusion criteria***:** Records with incomplete data.

The data was analysed as per the following microbiological parameters: primary smear, culture isolates and their antibiotic sensitivity. All laboratory procedures were performed, as per standard protocol. Microscopically, Gram-stained smear of wound swab was examined for presence of pus cells, Gram positive and Gram-negative organisms. For culture identification, a separate swab was inoculated on blood agar and MacConkey agar, within 30 minutes to 1 hour after collection and these are incubated at 35°C-37°C aerobically. They were examined for growth after 24hrs.

Culture identification was based on colony morphology, hemolysis on blood agar, lactose/ nonlactose fermenting colonies on MacConkeys agar, pigment formation, swarming, colour, odour etc. Gram-positive bacteria were further identified by testing biochemical tests such as catalase reaction, slide/ tube coagulase tests, bile esculin hydrolysis, bacitracin sensitivity etc. Gram-negative bacteria are identified by culture characteristics (eg lactose fermentation on MacConkeys agar, swarming, biochemical tests such as oxidase, triple sugar iron, motility, indole, citrate and urease tests. Antimicrobial susceptibility of the bacterial isolates by Kirby-Bauer disk diffusion method was performed and interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines.8 Antibiotic discs used for Gram negative organisms were: amoxicillin –clavulunate 20/10 µg, ampicillin – sulbactam10/10 µg, amikacin30 µg, aztreonam 30 µg, cefazolin 30 µg, cefuroxime 30 µg, ceftriaxone30 µg, cefotaxime30 µg, ceftazidime30µg, cefoperazone30µg, colistin /polymyxin 15 µg, doxycycline30 µg, gentamicin, tobramycin 10,10 µg, meropenem 10 µg and piperacillin –tazobactam 100/10 µg. Antibiotic discs used for Gram positive organisms were: cefoxitin30 µg, clindamycin 2 µg, cotrimoxazole1.25/23.75 µg, doxycycline30 µg,erythromycin (or azithromycin) 15 µg, linezolid 30 µg, penicillin10U, tetracycline30 µg and vancomycin30 µg.When interpreting oxacillin resistance in Staphylococcus species, all the plates were incubated for 24 hrs at 35°C. The diameters of the resultant zones of inhibition on each plate were measured in millimeters and interpreted as either sensitive, intermediately sensitive or resistant based on the criteria and breakpoints set by the CLSI, EUCAST and BSAC. All oxacillin resistant Staphylococcus aureus were considered to be methicillin resistant Staphylococcus aureus.

Gram negative isolates were tested for ESBL production using Mueller Hinton agar medium. The antimicrobial concentration of ceftazidime (CA) 30 ug, ceftazidime/clavulanic acid (CAC) 30/10 ug ; cefotaxime 30 ug cefotaxime/clavulanic acid 30/10 ug, were used for testing as per Kirby- Bauer Method and incubated for 16-18 hours at 35 deg C + 2 deg C. By using the disc combination method, CA/CAC were compared, for their ability to detect ESBL production phenotypically. The interpretation of results was as follows: A ≥5 mm increase in the zone diameter for either antimicrobial agent, tested in combination with clavulanate v/s the zone diameter of the agent when tested alone, was considered to be indicative of ESBL production. For the purpose of quality control Klebsiella pneumonia ATCC 25922 and E.coli ATCC 25922 were included as reference strains.[8,9]

RESULTS

In our study total 180 wound samples were studied. 100/180 (55%) samples showed growth of organisms on culture. Total number of culture isolates were 116. In 16/100 samples more than one (mixed) organisms were found.

The distribution of isolates shown in Table 1indicates that majority were Klebsiella (22%) followed by E.coli (20%), MRSA (17%), Pseudomonas (16%), S aureus (9%), Acinetobacter (6%) and others (10%) (eg Streptococcus, Morganella morganii, Proteus, Enterobacter, CONS, Citrobacter and Candida). Out of the total isolates, 70% constituted Gram-negative organisms and 30% were Gram positive organisms.

The study of antibiogram of Gram-negative organisms [Figure 1] revealed maximum 79% sensitivity to colistin/polymyxin, piperacillin/ tazobactum and aztreonam ;65% sensitivity to amoxicillin-clavulanate, gentamicin; 63% meropenem and 61% amikacin. High resistance was seen to: cefotaxime 88%, cefoperazone 83%, ceftriaxone, ceftazidime and cefuroxime 81% and cefazolin76%

The study of antibiogram of Gram-positive organisms [Figure 2] showed that majority 100% were sensitive to vancomycin, linezolid;90% to doxycycline and tetracycline and 75% to gentamycin. In our study 75 % Gram positive organisms were resistant to erythromycin.

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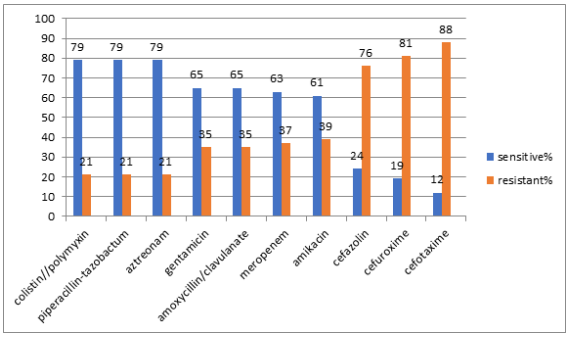

Figure 1: Distribution of isolates obtained from wound samples: The given pie chart depicts the proportion of isolates in the following format - (No. of Isolates; Percentage %).

With respect to multidrug resistant organisms: in our study, overall 20/116 ie 17.2% were MRSA; 20/31 ie 64% of Staphylococcus aureus were MRSA. Overall (31/49) i.e. 63% Gram negative isolates were multidrug resistant, ESBL producers; out of which (15/23) i.e. 67% Ecoli and (16/26) i.e.63% Klebsiella were ESBL producers.

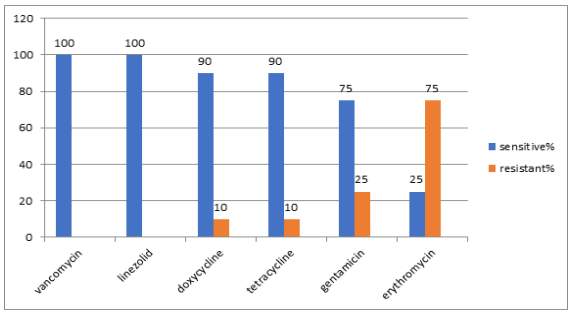

Figure 2: Antibiogram of Gram-negative isolates: The above graph depicts the comparative analysis of sensitive and resistant strains of Gram-negative isolates for the given profile of antibiotics.

Table 1: Distribution of Isolates: (n = 116)

Organism	No. Of isolates	Percentage %
Klebsiella	26	22.4%
E.coli	23	19.8%
MRSA	20	17.2%
Pseudomonas aeruginosa	19	16.3%
Staphylococcus aureus	11	9.5%
Acinetobacter	6	5.2%
Streptococcus,Morganella morganii Proteus, Enterobacter sp (2each)	8	6.9%
CONS, Citrobacter, Candida albicans (1 each)	3	2.6%

Table 2: Comparison of overall culture positivity from suspected samples

Our study	2022	Mumbai	55%
Roopshree et al.	2021	Bangalore	80%
Mohammed et al	2021	Bangladesh	72%
Wadekar et al.	2020	Karnataka	86%
Batra et al.	2020	Jaipur	85%
Narula et al.	2020	Uttarakhand	88%
S Mukherji et al	2020	Odisha	65%
Damen et al	2020	Nigeria	70%
Sania et al	2020	Pakistan	43%
Al Habsi et al	2020	Oman	58%
Pradeep et al.	2019	Andhra Pradesh	70%
Bhumla at al.	2019	Udaipur	89%
Pant et al	2018	Nepal	60%
Nithya et al.	2017	Tamil Nadu	71%
Ananthi et al.	2017	Chennai	60%

Table 3: Comparative Antibiotic sensitivity of Gram-negative isolates

Study	Most effective antibiotics
Our study	80%Colistin/polymyxin, piperacillin/ tazobactum, aztreonam; 65%-amoxicillin-clavulanate, gentamicin; 63% meropenem; 61% amikacin
Roopshree at al.	Imipenem, meropenem, amikacin
Ananthi at al.	85%Amikacin, 80%cefoperazone- sulbactum, 77% gentamicin
Wadekar et al.	Piperacillin tazobactum, meropenem
Nithya et al.	Amikacin,Piperacillin-tazobactum,ceftazidime,cefoperazone- sulbactum, imipenem
Pradeep et al.	(94%) Polymyxin B (75%) imipenem
Batra et al.	(22%) Imipenem, amikacin (6%) ceftazidime (15%) ciprofloxacin (94%) polymyxin, carbapenem
Narula et al.	Aminoglycoside, carbapenum
S Mukherji et al	Imipenem, meropenem, Piperacillin-tazobactum, Amikacin
Mohammed et al	Colistin, carbapenum, Piperacillin-tazobactum

Table 4: Comparative Antibiotic sensitivity of Gram-positive isolates

Study	Most effective antibiotics
Our study	(90-100%) vancomycin, linezolid, doxycycline, tetracycline
Ananthi at al.	(100%) vancomycin, linezolid, (93%) amikacin

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Wadekar et al.	(100%) vancomycin, (92%) linezolid
Nitya et al.	(100%) vancomycin, linezolid
Pradeep et al.	(100%) vancomycin, linezolid, (75%) tetracycline, (73%) gentamicin
Batra et al.	(100%) vancomycin, linezolid (92%) gentamicin (72%)
Narula et al.	(100%) vancomycin, (92-100%) linezolid, (78%) amikacin
Sania et al	(100%) vancomycin, linezolid

DISCUSSION

In our study the overall isolation rate was100/180 i.e. (55%) samples examined, showed growth of organisms on culture. Overall isolation rates of other studies, as compared to us is depicted in [Table 2].[10,11,12,13,14,15,16,17,18,19,20,21,22,23] Al Habsi et al had finding similar to us 58% while Sania et al had lower rate compared to us 43%.[17,18]

Distribution of isolates in our study was as follows: Klebsiella (22%) followed by E.coli (20%), MRSA(17%), Pseudomonas (16%), S aureus(9%). [Table 1] Thus the major pathogen was Klebsiella which was similar to Nithya et al where Klebsiella species was predominantly isolated (22.5 %). Most of the following studies reported Staphylococcus aureus as the major pathogen, Sowmya et al Chennai, Staphylococcus aureus (37.4%); Hariom et al, Staphylococcus aureus (31.58%); Bhumbla et al, S. aureus (26%); Wadekar et al, S. aureus (22.9%), Ananthi et al, Staphylococcus aureus (26.03%).[24,25]

The second major pathogen was E.coli(20%). In other studies where the second major pathogen were Gram negative organisms are as follows: Ananthi et al,[23] Escherichia coli (24.65%), Hariom et al,[24] Klebsiella pneumoniae (26.31%), Pseudomonas aeruginosa by Sowmya et al (29.6%); Bhumbla et al,[20] (22.4%), Wadekar et al,[12] (14.4%).

In our study *antibiogram of Gram negative organisms* [Figure 1] revealed maximum 79% sensitivity to colistin/polymyxin, piperacillin/ tazobactum and aztreonam;65% sensitivity to amoxicillin-clavulanate, gentamicin;63% to meropenem and 61% to amikacin. In comparision other studies reported various results as shown in [Table 2]. Higher amikacin sensitivity was also reported by Roopashree et al,[10] Ananthi at al,[23] Nithya et al,[22] and Batra et al.[13]

70% of the isolates constituted Gram negative organisms and 30% Gram positive organisms which was similar to Mohammed et al,[11] reported 86% Gram negative and 13 % Gram positive; Sania et al,[17] observed lower 42% Gram negative organisms as compared to 58 % Gram positive organisms.

In our study *antibiogram of Gram-positive organisms* showed that majority 100% were sensitive to vancomycin, linezolid;90% to doxycycline and tetracycline and 75% were sensitive to gentamycin. 90-100 % sensitivity to vancomycin and linezolid was reported by all studies as shown in [Table 3]. In our study 75 % Gram positive organisms were resistant to erythromycin and cefoxitin also reported by in other studies. Batra et al,[13] and Pradeep et al.[19]

In our study MRSA was isolated in 20/116 ie 17.2%. Somya et al,[24] Hariom et al,[25] Pant et al , Mohammed et al,[11] Wadekar et al,[12] and Nithya et al,[22] have reported higher rate 27%,30%,32%,33%, 48% and 41% respectively.

In our study Gram negative multidrug resistant ESBL producing organisms was noted in overall (31/49) 63%. Ecoli ESBL were (15/23) 67% and Klebsiella ESBL were (16/26) 63%. It was similar to Wadekar et al,[12] who reported overall ESBL producers as 61%. Mohammed et al,[11] reported lower incidence of 14% Gram negative ESBLs.

In our study ESBL production was higher in Ecoli 67% vs Klebsiella 63%. Nithya et al,[22] also reported higher Ecoli ESBL 60% v/s Klebsiella ESBL 56%. However other studies have shown higher ESBL in Klebsiella v/s E coli: Hariom et al,[25] 60% v/s50%; Somya et al,[24] 63% v/s 44%. Mohammed et al,[11] also reported Klebsiella as being the most common ESBL.

Preponderance of multidrug resistant Gram-negative bacteria in hospitalized patients in our study is a matter of concern. The growing antimicrobial resistance is a global threat with multidrug-resistant pathogens being rampant especially within hospitals. In depth study of associated risk factors need to be performed, periodic monitoring of resistance patterns among etiologic pathogens is crucial to control the spread to expedite wound healing and decrease cost and hospital stay.

Apart from antimicrobials recent advances have explored other technological/therapeutic options for skin regeneration such as wound dressings; skin substitutes; exogenous growth factor-based therapy and systemic therapy; external tissue expanders; negative pressure; oxygen; shock wave, and photobiomodulation wound therapies. Future trends in wound care which aim at novel formulations using metallic nanoparticles and topical insulin, herald promising therapeutic options that may change the wound care paradigm.[26]

CONCLUSION

Wound infection is one of the most common infections seen in patients admitted to surgical units. It is crucial to understand the antibiotic sensitivity profile of the etiological agents in these patients for specific therapy. The spectra of bacteria causing infections and their susceptibility pattern have been found to vary from one setting to another. Antibiograms aid to assess the susceptibility pattern of pathogens to various antibiotics and help tracking resistance trends. This also helps to keep a check on the antibiotic resistance patterns in the institution by

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incorporating pertinent changes in appropriate choice of antibiotic and prudent dosage administration. Eventually it can be part of a comparative study of antibiograms across a range of healthcare facilities.

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