Original Article

Isolation and Identification of Bacterial Pathogen in Wound Infection and Assess the Antimicrobial Susceptibility Pattern of the Isolates

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Abstract

Background: Wound infection is one of the health problems i.e. caused and aggravated by the invasion of pathogenic organisms. The presence of microorganisms within the wound causes local tissue damage and impedes wound healing. Infection can develop in any type of wound (surgical or trauma). The aim & objective is to isolate and identify bacterial pathogen in wound infection and assess their Antibiotic susceptibility pattern. **Material and Methods:** This study is to determine the identification and isolation of wound bacterial isolates present in the pus's swab and characterize their resistance profile to the most common antibiotics used in the therapy. This study was conducted among patients varies from different wards in Rama Medical College and Hospital and Research Centre in a defined period. Laboratory procedures including sample collection using sterile cotton swab and processed for bacterial isolation or culturing on various differential agar, Gram staining, motility, biochemical tests identify the species and antimicrobial susceptibility testing by using Kirby-Bauer disc diffusion method were performed during standard protocol. **Results:** Total 162 samples were collected, among these,78 (48.14%) showed growth and 84(51.85%) showed no growth. Out of 78 culture positive cases, 38 (48.7%) were males and 40 (51%) were female. Among these 78 bacterial isolates 38 were gram positive and 36 were gram negative and 4 fungal species. Staphylococcus aureus (41%) was the most frequent organisms followed by Klebsiella species (12.8%), Escherichia coli (12.8%), Pseudomonas species (10.2%), Proteus species (7.69%), CONS (5.12%), Citrobacter species (2.56%), Acinetobacter species (2.56%). Staphlyococcus aureus being the most dominant isolate and was most susceptible to Vancomycin, Menocycline and Linezolid. **Conclusion:** Routine microbiological analysis of wound specimen and their AST are recommended that will guide medical practitioners for imperical treatment of wound infection, to reduce the spread of resistant of bacterial isolates. It is essential to do any surgical procedure in aseptic manner.

Keywords: Pus sample, Wound infection, AMR (Antimirobial resistance). Gram positive cocci, Gram negative bacilli, MDR.

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INTRODUCTION

The primary function of intact skin is to control microbial populations that live on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens.[1] Surgical infection is one of the most important causes of morbidity and mortality worldwide and antibiotics resistant bacteria are the great part of concern.[2] Wound injuries are the most common and serious types of trauma and represent a major public health concern. There are many potential factors (age, sex, diabetes, stress, nutrition, and oxygenation) involved in the complex wound healing process that can delay healing. Breach intact skin surface whether it is caused by trauma, accident, surgical operation or burn provides an open door for bacterial infection.[3] Because of localized inflammation, there is pus formation which consists of white blood cells, damaged cells and dead tissues.[4] Exposure of the subcutaneous tissue following loss of skin integrity provides an environment that is moist, warm, and nutritive all of which are conductive for microbial colonization and proliferation.[5] Depending on the cause, site and depth of a wound can lead from simple to severe one including open or closed, acute or chronic, clean or contaminated, internal or external, non-penetrating wounds and penetrating wounds.

The risk of wound infection increases with the degree of contamination and it has been estimated that about 50% of wounds contaminated with bacteria become clinically infected.[6] An economic retrospective analysis of medicare beneficiaries identified that 8.2 million people had wounds with or without infection.[7] Sepsis affected 49 million people and was linked to approx. 11 million deaths worldwide roughly 20% of annual global deaths. 20 million of all estimated sepsis cases worldwide and 2.9 million deaths occur in children under 5 in 2017.[8] Risk factors includes poor blood circulation, diabetes,

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obesity, compromised or suppressed immune system, decreased mobility or immobility, malnutrition and poor hygiene. Approx. 2% of all hospitalized patients worldwide have a chronic wound and older adult patients are at higher risk, because aging impairs the healing process.[9] The most common pathogenic organisms associated with wound infection includes Staphylococcus aureus / MRSA (Methicillin resistant Staphylococcus aureus), CONS (coagulase negative Staphylococcus aureus), Pseudomonas species, Escherichia coli, Proteus species, Candida species, Klebsiella species, Citrobacter species, Acinetobacter species.

Materials and Methods

Inclusion Criteria

The Pus samples collected from the patient attending outpatients (OPD) and inpatients (IPD) Departments of Rama medical college and hospital. Determined by treating physician were included in this study.

Exclusion Criteria

1. Repeat sample of same IPD and OPD patients are excluded.
2. Same Antibiotic Susceptibility Pattern.

This prospective study was conducted in Rama Medical College, Hospital and research centre, Hapur over a period of 6 months. The clearance was taken by the institutional ethical committee, verbal and written informed consent were taken from all the subjects before study participation at department of microbiology. Total 162 samples were collected from both male and female patients of age 2-72 years. Patients present at the outpatients departments (OPD) and inpatients departments (IPD) were included in this study. Clinical samples were collected from pus, tissue materials and discharged from the incised lesions. Aseptically the swab stick was rotated over 1cm² area for 5 sec with sufficient pressure to express fluid and bacteria to surface from within the wound tissue. The wound samples were transported to Microbiological Laboratory after collection in 0.5ml sterile normal saline solution for bacterial preservation. The wound swab samples were cultured by plating onto Blood agar plates and MacConkey agar and incubated aerobically at 37° C for 48 hours. Suspected colonies were further subculture to obtain discrete colonies. The isolated bacteria were identified by macroscopic and microscopic observations (Gram staining). Gram staining was conducted as a preliminary test. Pure culture isolated, was identified based on morphological appearance on blood agar and MacConkey agar, motility, biochemical tests (citrate, catalase, coagulase, oxidase, indole, methyl red, urease, triple sugar iron).[10,11] Antibiotic susceptibility of bacterial isolates were performed by Kirby-Bauer disk diffusion process. After that antibiotic discs were placed on the surface of media and pressed gently. Mueller Hinton agar was then incubated at 37°C for 24 h. After 24h the inhibition zones were measured and interpreted by the recommendations. of Clinical and Laboratory Standards Institute, (CLSI).[12] Data were analysed using SPSS version 20 software. The chi-square test was used for statistical analysis.

Results

In this study, the total 162 samples were collected; of which 78 showed bacterial growth. Among these 66 were males and 96 were females. The remaining 84 had no growth. Out of total 78 bacterial isolates, 38 were Gram negative and 36 were Gram positive and 4 fungal species. Staphylococcus aureus were most frequently isolated bacteria accounting for 41% followed by Klebsiella species (12.8%), Escherichia coli (12.8%), Pseudomonas species (10.2%), Proteus species (7.69%), Candida species (5.1%), Acinetobacter species (2.56%), and Citrobacter species (2.56%).

Table 1: Distribution of total sample according to age and gender

Age in years	Male (n)	Female (n)	Total
0-20	10	14	24
20-40	30	40	70
40-60	16	38	54
60-80	10	04	14
Total samples	66	96	162

Table 2: Prevalence of pathogen of wound infection

Bacterial isolates	Patients (n)	Percentage (%)
S. aureus	32	41
Klebsiella	10	12.8
E. coli	10	12.8
Pseudomonas spp.	8	10.2
Proteus spp.	6	7.6
Candida spp.	4	5.1
CONS	4	5.1
Citrobacter spp.	2	2.5
Acinetobacter spp.	2	2.5
Total	78	100

Table 3: Prevalence of infection in male and female according to their age

Age in years	Male with infection		Female with infection	
	N	%	N	%
0-20	6	15.7	6	15
20-40	14	36.8	22	55
40-60	12	31.5	12	30

60-80	6	15.7	0	0
Total	38	100	40	100

Table 4: Antibiotic resistance profile of GPC (all values are in percentage %)

Antimicrobial tested	MRSA	MSSA
Vancomycin	-	-
Tetracycline	37.5	25.0
Gentamicin	50.0	31.2
Erythromycin	56.2	34.3
Ciprofloxacin	62.5	50.0
Clindamycin	25.0	18.7
Menocycline	25.0	21.8
Pencilline	87.5	84.3
Levofloxacin	37.0	31.2
Linezolid	31.2	40.6
Co-trimoxazole	43.7	31.2
Cefoxitin	14.2	-

Table 5: Antibiotic resistance profile of GNB (all values are in percentage %)

Antibiotic tested	E. coli	Klebsiella	Pseudomonas	Proteus
Gentamicin	40	40	-	32.3
Ciprofloxacn	70	80	75.0	66.6
Amoxyclave	50	60	-	83.0
Co-trimoxazole	50	60	-	50.0
Imipenem	30	40	37.5	-
Meropenem	10	20	25.0	-
Ertapenem	40	30	-	33.3
Amikacin	20	40	-	12.9
Ceftriaxone	50	60	-	33.3
Cefuroxime	40	20	37.5	66.6
Levofloxacin	20	40	50.0	66.6
Cefazolin	50	60	-	66.6
Ceftazidime	30	40	25.0	-
Piperacillin	10	30	16.5	-
Cefotaxime	20	20	-	16.0
Doripenem	20	40	-	-
Tobramycin	20	40	25.0	33.3
Colistin	-	-	-	-
Aztreonam	50	40	25.0	-
Cefepime	40	60	16.4	66.6
Tetracycline	80	40	-	-

Discussion

The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to greater incidence of infections caused by MDR pathogens like ESBL, MBL. Out of 162 samples (48.14%) showed bacterial growth which is in concordance with Jamatia et al. of which shows (49.02%) growth.[13] But lower than the study done by Goel et al (71.5%), Rao et al. (89.4%), and Sharma et al (83%).[14] The higher no. of bacterial growth was observed in inpatients. The highest positive cases (46.15%) were observed in age group (21-40) year. Among the total bacterial isolates 48% were gram negative and 46% were gram positive which is in the agreement with studies done in Zaria, Nigeria.[14] Major organisms isolated from infected wounds were S. aureus (41%) in pus sample which is in the agreement with the study by Rao et al.[15] In our study E.coli and Klebsiella spp. were the second most common isolates but in another study by Basu et al,[16] reported Pseudomonas and E.coli to be most commonly occurring pathogens in wound infection. Linezolid, Menocycline, Vancomycin used in this study was to be more sensitive to GPC whereas Meropenem, Imipenem, Ertapenem, Amikacin, Piperacillin was found more sensitive for GNB which showed some similarity with the study of K.Prabhat et al. and Sarvan Ricky R et al.[17,18] The findings underscore the critical need for regular surveillance of antimicrobial susceptibility patterns. The high resistance rates observed in this study suggest the necessity for cautious antibiotic use and the importance of adopting antibiotic stewardship programs to curb the spread of resistant strains. Personalized treatment strategies based on local antibiograms are essential to improve patient outcomes and reduce the incidence of treatment failures (Alharbi et al. (2022) and Sewunet et al. (2013).[19,20]

Conclusion

Routine microbiological analysis of wound specimen and their AST are recommended that will guide medical practitioners for imperical treatment of wound infection, so as to reduce the spread of resistant of bacterial isolates. It is essential to do any surgical procedure in aseptic manner. Continuous surveillance is necessary to guide appropriate therapy for wound infections and rational use of antimicrobial agents should be sought to prevent the MDR pathogens.

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Conflicts of interest

There are no conflicts of interest.

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