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Research Paper

Isolation and Identification of Wound Pathogens and Analysis Antibiotic Sensitivity Using Antibiotics and Soaps

Chemistry

KEYWORDS : Wound, Bacteria, SDS-PAGE, Antibiotic sensitivity

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ABSTRACT

In the present study 25 wound swabs were collected from burnt patients in Tiruchirappalli district Tamil Nadu. From the swabs bacterial species were isolated using Nutrient agar, MacConkey and Blood agar medium. The bacterial isolates were identified with the help of culture, morphological and biochemical characteristics. The bacterial isolates were confirmed as Streptococcus pyogenes, Proteus vulgaris, Pseudomonas aeruginosa and Staphylococcus aureus. The isolated bacterial species protein profile was also analyzed by SDS-PAGE. Seven bands were observed in Streptococcus pyogenes band range from 160-36 KDa. In bacteria, the maximum and minimum zone of inhibition was observed in antibiotic ceftriazone and clindamycin respectively. The highest antibacterial activity was noted De'scab, Deltol, and Lifebouy against all bacterial isolates. At the same time Lifebouy highly inhibit the growth of Proteus vulgaris (16±0.81 mm in diametery).

INTRODUCTION

Wound infection occurs due to contamination colonization, and multiplication of microorganism in wound. Wound contamination is the presence of bacteria within the wound which do multiply or initiate a host reaction. Wound colonization is the presence of bacteria within the wound which do multiply or initiate a host reaction (Ayton, 1985). Burn wound infections are one of the most important and potentially serious complications that occur in the acute period following injury (Beetstra, 1986; Appelgren *et al.,* 2002). Bacteria rapidly colonize open skin wounds after burn injury. Microorganisms colonizing the burn, wound originate from the patients endogenous skin and gastrointestinal and respiratory flora (Manson *et al.,* 1992; Erol *et al.,* 2004). The burn wound surface (in deep partial thickness and in all full-thickness burns) is a protein rich environment consisting of a vascular necrotic tissue that provides a favorable rich for microbial colonization and proliferation (Barret and Herndon, 2003).

Burn wound sepsis was predominantly due to invasive wound infection prior to the advent of early burn wound excision (Gray *et al.,* 1982; Thompson, 1987). Wide spread application of an effective topical antimicrobial agent substantially reduces the microbial load on the open burn wound surface and reduce the risk of infection (Manofo and West, 1990; Heggers *et al.,* 2002). By controlling infection, effect topical antimicrobial therapy decreases the conversion of partial-thickness to full thickness wounds, but its use in adjunctive to early excision therapy. Section of topical antimicrobial therapy should be based on the agent's ability to inhibit the microorganism recovered from burn wound surveillance cultures and monitoring the nosocomial infection acquired in burn unit. Keeping the above view in mind the present isolation and identification of wound pathogens and analysis antibiotic sensitivity using antibiotics and soaps

MATERIALS AND METHODS

In this study 25 burn wound swab samples were collected from different hospital in Tiruchirappalli. Bacterial species were isolated from the collected burn wound swab sample using Nutrient agar, MacConkey agar and Blood agar by streak plate method. After the streaking the bacterial plates were incubated at 37˚C for 24-48 Hrs. After incubation Cultural characteristics and colony morphology were observed. These colonies were sub cultured and stored in refrigerator for further study.

The morphological and biochemical tests were conducted by the following methods as described by Norris and Ribbons, (1972) to identify the bacteria. Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis is an excellent method for rapidly assessing the purity of proteins and is routinely used in the development and validation of purification strategy. The sample was then subjected to SDS-PAGE (Sodium Dodecyl Sulphate- Polyacrylamide gel electrophoresis).

The commercially available antibiotic disc such as Amikacin, Ciprofloxazin, Ampicillin, Gentamycin, Tetracycline, Ceftriaxone, Erythromycin, Carbenicillin Clindamycin and Piperacillin used for bacterial culture. The antibiotic disc were purchased from Hi media chemical Pvt. Ltd, Mumbai. The commercially available soaps such as Tezesol, Flobact, De'Scab, Dettol, Savlon and Lifebuoy were purchased from medical shop, Tiruchirappalli.

The antibiotic sensitivity of isolated bacterial species to the commercial antibiotic tests was analyzed by disc diffusion method (Bauer *et al.,* 1996). The isolated bacterial strains such as *Streptococcus pyogenes, Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa* cultures were swabbed on the Muller-Hinton agar medium separately. After this swabbing different commercial antibiotic discs were placed on the each culture swabbed plate. The plates were incubated at 37˚C -24 hrs for bacteria, after the incubation period zone of inhibitions were noted.

The commercial soap antibacterial activity was analyzed against bacterial isolates by agar well diffusion method (Wasn, *et al.,* 1998). The Muller-Hinton agar medium was poured onto the sterile petriplates and allowed to solidify. After solidification the sterile glass stems (diameter 6 mm) were placed on the medium, then the glass stems were removed and obtained wells were filled with different commercial soap solution respectively of same concentration (500µl).

RESULTS

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In the present study, four different bacterial colonies were noted. The colonies were named as WB1, WB2, WB3 and WB4. Most of the isolates were Gram positive cocci where as WB2 and WB4 were Gram negative rod. The isolate WB2 showed indole, MR, Catalase test positive whereas WB4 showed indole, MR, VP, Catalase and urease test negative. The isolate WB1 showed negative for all biochemical tests done except methyl red and carbohydrate fermentation. The finding results were compared with Bargey's manual of systematic bacteriology, based on the comparison the isolated bacterial strains such as WB1, WB2, WB3 and WB4 were confirmed as *Streptococcus pyogenes, Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively (Table – 1).

The percentage of bacteria isolated from 25 burn wound patients were presented in (Fig - 1). About 56% of patients were affected by *Pseudomonas aeruginosa* and it was the pre dominant organisms in burn wound patients. Whereas *Proteus vulgaris* and *Streptococcus pyogenes* caused infection in only 12% of the patients. *Staphylococcus aureus* was the second major microbe caused infection in 20% of the patient.

The isolated bacterial species were identified with the help of protein profile the results (Fig – 2). Among this study different molecular weight protein bands were noted in each bacterial isolates. Seven bands were observed in *Streptococcus pyogenes* band range from 160-36 KDa. Similarly 5, 8,7 and 7 bands were observed in *Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa* bands range from 110-21, 105-34 and 90-12 KDa respectively.

In the present study maximum antibacterial activity were noted in Ciprofloxacin when compared to others. At the same time lowest antibacterial activity was noted in Ampicillin. *Proteus vulgaris* is mostly resistant to antibiotics at the same *Streptococcus pyogenes* and *Staphylococcus aureus* is more sensitive to antibiotics (Fig – 3). In the highest antibacterial activity was noted De'scab, Deltol, and Lifebouy against all bacterial isolates. At the same time Lifebouy highly inhibit the growth of *Proteus vulgaris* (16±0.81 mm in diametery). Moderate antibacterial activity noted in Tezezol and Savlon (Fig – 4).

DISCUSSION

In the present study finding that *P. aeruginosa* was the most common isolate and it was the predominant species in causing burn wound infection coincides with previous report Agnihotri *et al.,* (2004) explained that *Pseudomonas aeruginosa* was the common organisms in causing wound infection. *Pseudomonas aeruginosa* caused infection in 48% of burn patient. This study clearly showed that Pseudomonas species were the most commonly isolated organisms amongst the gram negative bacilli. This is however contrary to the observations of Mordi *et al.,* (2001) who claimed that protease species were the predominant in burn wounds at 44.64% followed by *Staphylococcus aureus* (30%) and Pseudomonas as the third prevalent Again Kehinde *et al.,* (2004) working in hospital in Ibadan observed prevalence rates of *Klebsiella* speices were most pre-dominant in burn wounds at 34.4% where as *Pseudomonas* species is 29%.

The present study *Staphylococcus aureus* was the second predominant strain that causes infection in the patients of about 20% where as *E. coli* and *Streptococcus pyogenes* was the least prevalent in causing infection of about 4%. Ahmad *et al.,* (2006) in their study have demonstrated that infections by gram positive organisms were more common in first 5 days of burns while gram negative organisms dominant the infection scene thereafter. But in our study were did not find much difference in the pattern of organisms isolated.

Antibiotic resistance due to inappropriate use of drugs is common finding in our environment. Both medical and paramedical staff must be educated regarding the rational use of antibiotics similarly, the community at large must enlightened. Through proper health education programmes about the dangers inherent in self medication. Alireza, (2007) demonstrated that *Pseudomonas aeruginosa* was resistant to gentamicin cephalotoxime, Ciprofloxin, Amikacin, Carbnicillin, Ceftazidime to bramycin (100%) and aphalexin (98%) respectively. In this review *Pseudomonas aeruginosa* was resistant against Ampicillin, Erythromycin, Gentamycin and Tetracycline whereas sensitive against Ciprofloxacin, Pipercillin, Carbencillin and Amikacin. In this study gram negative organisms showed resistant against Erythromycin, Ampicilline, Ceftriaxone and Carbencillin.

Finally it is quite clear that infections are serious problem among burn patients. *Pseudomonas aeruginosa* has emerged as the commonest organisms causing infection and *Escherichia coli* isolated is resistant to most of the antibiotics. To keep a check on burn wound infections it is important for every hospital to have a data on prevalent organism and their susceptibility pattern. This study should be done frequently to check the changing pattern of the organisms and their susceptibility pattern. Based on this the hospital should formulate an effective antibiotic policy.

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Table – 1. Biochemical Characteristics for Isolated Bacteria

Organisms	Gram staining	Motility	Indole	MR	VP	Catalase	Oxidase	Citrate	Urease	TSI	Sugar fermentation		
											D	L	S
WB1	G(+) cocci	-	-	+	-	-	-	-	-	K/K	A	A	A
WB2	G(-) Rod	+	+	+	-	+	-	-/+	+	K/A	A	-	-
WB3	G(+) cocci	-	-	+	-/+	+	-	-	-	K/A	A	A	A
WB4	G(-) Rod	+	-	-	-	-	+	+	-	K/K	A	-	

+ - positive
+ - Negative
± - variable
A- Acid
A/A – Acid slant and acid butt
K/K – Alkaline slant and alkaline butt
K/A – Alkaline slant Acid butt
WB1 - *Streptococcus pyogenes*
WB2 - *Proteus vulgaris*
WB3 - *Staphylococcus aureus*
WB4 - *Pseudomonas aeruginosa*

Fig – 1. Bacterial Infection in Burn Wound (%)

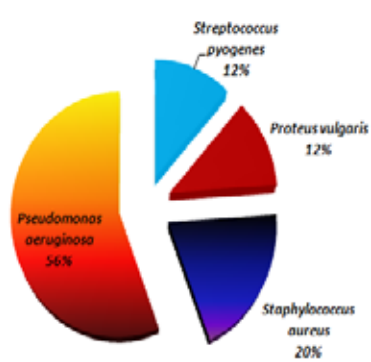
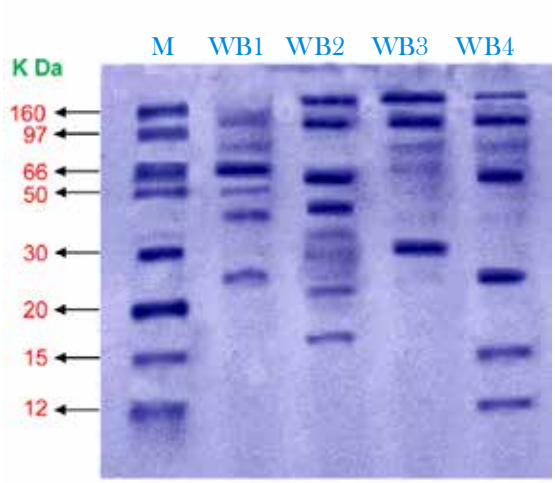


Fig - 2 Protein Profiles of Isolated Bacteria



WB1 - *Streptococcus pyogenes*
WB2 - *Proteus vulgaris*
WB3 - *Staphylococcus aureus*
WB4 - *Pseudomonas aeruginosa*

FIG – 3. Assay of Anti Bacterial Activity using antibiotics

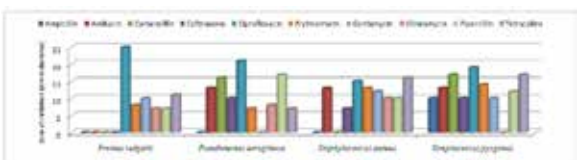
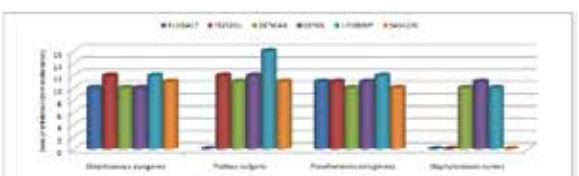


Fig – 4. Assay of Antibacterial Activity using soaps



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