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Microbial Assessment of Wound Infection and Antimicrobial Receptiveness Against *Pisidium gujava, Citrus Limon*

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Abstract

A wound is a break in the tissues of the body. Wounds are mainly classified into two types. They are open wounds and closed wounds. The breakage which cause in the external part of the body is termed as open wound and the breakage which cause in the internal part of the body is termed as closed wounds. Wound infections and abscesses occur as complications of surgery, trauma or diseases that may interrupt a mucosal or skin surface. The nature of the infecting flora depends on the underlying problem and the location of the process. Surgical site infections (SSI) is defined as an infection occurring within 30 days after a surgical operation (or within 1 year if an implant is left in place after procedure) and affecting either incision or deep tissues at the operation site. This infection may be superficial or deep incisional infection or infections involving organ or body space. Postoperative SSI is among the most common problems for patients who undergo operative procedures and the third most frequently reported nosocomial infection in the hospital population. Postoperative surgical site infections are associated with increased morbidity, mortality, prolonged hospital stay and increased economic costs for patient care. *Psidium guajava, Citurs limon* zone of inhibition in mm is 11, 19 and 13, 21 and 12.1, 21 and 10, 18 for *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae.*

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INTRODUCTION

Staphylococcus sp, colonise prosthetic implants as a biofilm, multiple layers of sessile cells that adhere to the implant surface as well as to each other. Once a biofilm has formed, it can be very difficult to treat clinically because the bacteria from the host immune response as well as antibiotic agents. Vancomycin remains the drug of choice for treatment of infections due to methicillin resistant *Staphylococcus sp, Staphylococcus sp,* is the leading cause of nosocomial infections. More than 50% of these infections are caused by methicillin resistant *Staphylococcus sp,* Klebsiella *sp,* is not an intracellular pathogen and hence iron is not readily accessible to this organism in the body. To circumvent there in vivo iron restriction *Klebsiella sp,* produces siderophores i.e enterochelin and aerobactin, which are capable of removing iron from host iron binding proteins. Siderophores are the soluble, low molecular mass high affinity iron-chelating compounds produced by organism [1-3]. Sacchetti *et al.* reported that the oil showed a strong resistance against *Yarrowia lipolytica* which is a pathogenic yeast. Vieira *et a* have also reported the antibacterial effect of guava leaves extracts and found that they inhibited the growth of the *S. aureus.* Gnan and Demello (testing guava leaf extract found good antimicrobial activity against nine different strains of *Staphylococcus aureus* [4-7]. The antibacterial activity of guava leaf extract was tested against acne developing organisms by Qa'dan *et al.* Concluding that the leaf extracts may be beneficial in treating acne especially when they are known to have anti-inflammatory activities [8-10]. The lemon peel extracts are done by different solvents such as ethanol, methanol and acetone which are subjected to antibacterial assay. Methanolic extract shows higher antimicrobial activity against tested microorganisms (*E. coli, S. aureus, Candida albicans and Trichophyton rubrum*).

MATERIALS AND METHODS

Site of Collection

The samples for bacteriological screening were taken from government hospital, Kumbakonam, Thanjavur (DT). Where several patients with accidental wounds, burns, surgical wounds and pressure sore. The sample collected by swab and transport to the microbiology lab for the present investigation.

Bacteriological Analysis

The collected sample is then enriched in enrichment medium. From this the culture is transferred into a selective medium. The selective media used for isolation of *Staphylococcus sp,* is mannitol salt agar. The selective media used for isolation of *Streptococcus sp,* is blood agar. *Pseudomonas sp,* was isolated using *Pseudomonas* isolation agar as selective medium. *Escherichia coli* was isolated using Eosin methylene blue agar as selective medium. *Klebsiella* sp, was isolated using Macconkey agar as selective medium.

Microscopic Examination

Hanging Drop Method

Motility of bacteria is identified using a loopful of overnight broth culture of the isolate by hanging drop method.

Gram's Staining

A smear of suspected colony was made on a clean glass slide and heat fixed. It was flooded with crystal violet solution and allowed to remain for one minute. Then it was washed with water flooded with iodine solution and allowed for one minute. It was then drained and decolourised with 95% ethanolfor 15-30 seconds and then washed and counterstained with saffranin for one minute, then examined under microscope.

Biochemical Tests

Indole Production Test, Methyl Red Test, Voges Proskauer Test, Citrate Utilization Test, Triple Sugar Iron Agar Test, Gelatin Hydrolysis, Starch Hydrolysis, Catalase Test, Urease Test, Lipid Hydrolysis, Coagulation Test

Antibiotic Sensitivity Test

Sterile Muller Hinton agar plates were prepared and the plates were swabbed neatly with the isolated cultures. A few antibiotic discs were selected and placed on the media using sterile forceps. Care was taken that the distance between the two discs placed on the media should be at least 25 mm apart. Hence the zone produced by two adjacent antibiotic discs cannot co-incide each other. All the plates were incubated at 37°C for 28 hours. After incubation, the diameter of the zone was measured in millimetres for each antibiotic disc and the values were recorded. The recorded values were interpreted using standard reference. The antibiotics used in this study were lincomycin, ciprofloxacin, amikacin, gentamycin, chloramphenicol, norfloxacin, tobramycin, cotrimoxazole, vancomycin and cefotamine.

RESULT

The surgical wound material on smearing and culturing helped to determine the type of organisms culturing, isolation, identification and characterization by sensitivity test with antibiotics and disinfectants are followed. About seventy samples were collected from various types of

wounds for this study. Table.1 shows prevalence of bacterial flora in the various types of wounds. *Staphylococcus aureus* is appearing gram positive, coccus shape. Its appear like agar slant cultural characteristics is abundant opaque golden growth. Gelatin liquification is positive, starch hydrolysis is negative. Lipid hydrolysis is positive, lactose is producing acid. Dextrose is produced acid. Sucrose produces acid form. H_2S production is negative. No3 reduction is a positive, indole production is negative, MR reaction is positive, VP reaction appear positive or negative, citrate is a negative, urease is a negative, catalase is a negative, oxidase is a negative. Pseudomonas *aeruginosa* is appear gram negative, rod shape. Its appear like agar slant cultural characteristics is abundant in thin white medium turns. Gelatin liquification is positive, starch hydrolysis is negative. Lipid hydrolysis is positive, lactose is negative. Dextrose is negative. Sucrose negative. H2s production is negative. No3 reduction is a positive, indole production is negative, MR reaction is negative, VP reaction appear negative, citrate is a positive, urease is a negative, catalase is a positive, oxidase is a positive. *E.coli,* is appear gram negative, rod shape. Its appear like agar slant cultural characteristics is white, moist, glistening. Gelatin liquification is negative, starch hydrolysis is negative. Lipid hydrolysis is negative, lactose is produce acid and gas. Dextrose is produce acid and gas. Sucrose produce acid and positive or negative gas. H2s production is negative. No3 reduction is a positive, indole production is positive, MR reaction is positive, VP reaction appear negative, citrate is a negative, urease is a negative, catalase is a positive, oxidase is a negative. The testing antimicrobial activity of *Staphylococcus sp,* on *Psidium gujava*, *Citrus limon.* In *Citrus limon* maximum zone of inhibition (19mm) observed, minimum zone of inhibition (10mm) observed, in *(*15mm), *Psidium guajava* (11mm) were observed. The testing antibiotics sensitivity test, of isolated microbes *staphylococcus, Pseudomonas, Klebsiella, E.coli* against Penicillin, Tetracycline, Erythromycin, Amphicillin. In *staphylococcus* the maximum zone of inhibition (16mm) observed in erythromycin and minimum (10 mm) in penicillin. In tetracycline (12.5mm) and tetracycline (12mm). In *Pseudomonas* antibacterial activity tested against antibiotics disc the maximum zone of inhibition (14 mm) observed in Amphicillin, minimum zone of inhibition observes (11 mm) in Penicillin. In Erythromycin (13.6 mm), and Tetracycline (13mm) zone of inhibition observed. In *Klebsiella sp.* Antibacterial activity tested against antibiotic disc, Penicillin, Tetracycline, Erythromycin, Amphicillin, the maximum zone of inhibition observes (17mm) in Amphicillin minimum in Erythromycin (12). In Tetracycline (16mm) and Penicillin (15mm) Zone of inhibition was observed. In *E.coli.* testing antimicrobial activity against Penicillin, Tetracycline, Erythromycin, Amphicillin, the maximum zone of inhibition (15mm) in Erythromycin minimum observed (17mm) observed in Tetracycline. In Amphicilin (19mm), and Penicillin (18mm) were observed.

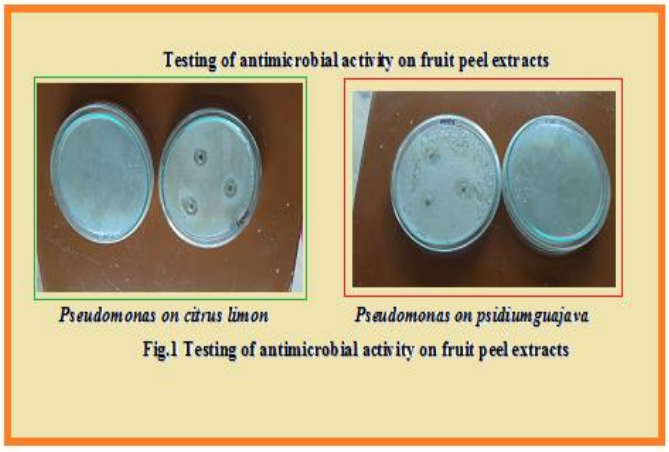

Fig.1 Testing of antimicrobial activity on fruit peel extracts

Table-1 Types of wound and percentage of sample collection.

Types of wound	No.	Percentage
Accidental	20	100
Surgical	13	100
Burns	1	100
Pressure sore	1	100

Table-2 Prevalence of bacterial flora in wounds:

Types of wound	Bacterial flora	No	Percentage
Accidental	*Staphylococcus aureus*	*36*	51.3
Surgical	*Pseudomonas aeruginosa*	*23*	32.8
Burns	*Escherichia coli*	*9*	12.7
Pressure sore	*Klebsiella pneumoniae*	*39*	55.1

Table-3 Testing antimicrobial activity of *Staphyloccus Sp.,*

S.No	Fruits extract	Zone of inhibition (mm)	S/R
1	Psidium guajava	11	S
2	Citurs limon	19	S

Table-4 showing antimicrobial activity of *Pseudomonas sp.,*

S.No	Fruits extract	Zone of inhibition (mm)	S/R
1	Psidium guajava	13	S
2	Citurs limon	21	S

Table-5 Showing antimicrobial activity of *E.coli sp.,*

S.No	Fruits extract	Zone of inhibition (mm)	S/R
1	Psidium guajava	12.1	S
2	Citurs limon	21	S

Table-6 showing antimicrobial activity of *Klebsiella sp.,*

S.No	Fruits extract	Zone of inhibition (mm)	S/R
1	Psidium guajava	10	S
2	Citurs limon	18	S

TABLE-7: ANTI BIOTIC SENSITIVITY TEST

S.No	Microbial Species	Zone of Inhibition (mm)			
		Penicillin	Tetracyclin	Erythromycin	Amphicilin
1	*Staphylococcus sp,*	S (10mm)	S (12mm)	S (16mm)	S (12mm)
2	*Pseudomonas sp,*	S (11mm)	S (13mm)	S (13mm)	S (14mm)
3	*Klebsiella sp,*	S (15mm)	S (16mm)	S (12mm)	S (17mm)
4	*E.coli sp,*	S (18mm)	S (17mm)	S (15 mm)	S (19mm)

S – Sensitive R –Resistant

CONCLUSION

The study presents a clear understanding to the causative pathogens of wound infections in this hospital and their sensitivity and resistance profiles. It has been concluded that wound infections in this were polymicrobic in nature and, in most cases, associated with *S. aureus,* coagulase negative *staphylococcus*, *E.coli* and *Pseudomonas aeruginosa*. Results also displayed that there is a high rate of antibiotic resistance in all pathogens isolated. Of all the antibiotics tested, vancomycin was shown to be the one most likely to be effective in treating infections as, in contrast to other antimicrobial agents tested in this study, not a single bacterial isolate was found to be resistant to its activity. In the present investigation carried out isolated microbes from accidental wound the microbes *Staphylococcus sp., Pseudomonas sp., E.coli sp., Klebsiella* isolated from various wound infection and testing its antibiotic activity agent Tetracycline, Penicillin, Amphicilin, Erythromycin and juice extract of Psidium guajava, Citrus limon. In spite of technological advances that have been made in wound management, wound infection has been regarded as the most common nosocomial infection. A wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity which provides a moist, warm, and nutritive

environment that is conducive to microbial colonization and proliferation. Automobile accident wound infection simply means wound infection after automobile accident. A wound can be considered infected if purulent material is observed without the confirmation of a positive culture. Infection in a wound delays healing, causes wound breakdown, dehiscence prolongation of hospital stays, increased trauma care and treatment cocts. The number and range of automobile accidents had increased over the recent decades and the rates of automobile accident wound infection vary from one patient to another. Bacteriological studies have also shown that wound infection is universal and that the bacteria types present vary with geographical location, bacteria resistant on the skin, clothing at the site of wound time between wound and examination. In the present work from accidental wound *Klebsiella, Staphylococcus, Pseudomonas, E.coli* isolated. The commonest aerobic isolate from pyogenic infections was *Pseudomonas aeruginosa* found along with *Clostridial* group from 5 samples next being *Staphylococcus aureus* with *prevotella.* This work agreed with and *Klebsiella pneumonia* was the most frequent isolate causing surgical wound infection, followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. All the isolates were resistant to the commonly used antimicrobial agents. The microbiologicl analysis revealed that S. aureus was the leading etiologic agent of wound infection and the results obtained in, India, Thailand and japan. The high prevalent of S. aureus must have been caused by incision or fluid collection under the skin surface. Microbiological investigations have noted that this organism is the single causative bacterium in approximately 25 to 69% of cutaneous abscess. The emergence of resistant organisms in automobile accident wound infections can lead to higher treatment costs and prolongation of hospital stay with serious consequences in infection control especially in developing countries. Most of the wound infections were polymicrobic in nature and in most cases, associated with S. aureus and other microorganisms. The postoperative wound infection is the commonest nosocomial infection only after the urinary tract infection. The present microbiological study has determined the commonest bacteria responsible for the post-operative wound infections. There was predominance of commonly isolated bacterial species were S. aureus, P. aeruginosa and E.coli. Hence the study concludes that the strict majors need to be taken to avoid such post-operative wound infections. Also the treatment of this may leads to the antibiotic resistance. The data in the present study may be useful in the for choosing the effective therapy against the isolates from postoperative infected wound. The increasing failure of chemotherapeutic and antibiotic resistant exhibited by pathogenic microbial infection agents has leads to the screening of the several medicinal plants for their potential antimicrobial activity. In our findings *Citrus limon* shows maximum zone of inhibition (19mm) against *Staphylococcus sp,* (21mm) against *Pseudomonas sp, (*18mm) against *E.coli* (18mm) against *Klebsiella. Citrus limon* show maximum zone of inhibition among the 4 plants *Psidium guajava, Citrus limon.*

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