Bacterial spectrum in surgical wound infection, its susceptibility pattern and biofilm formation among isolates

Siddesh B. Sirwar[1], Nazneen Fatima[2,*]

[1]Professor and HOD., [2]Post Graduate Student, Dept. of Microbiology, KBNIMS., Kalaburagi, Karnataka, India

***Corresponding Author:**
Email: nazneenf105@gmail.com

Abstract

Topic: Bacterial spectrum in surgical wound infection, its susceptibility pattern and biofilm formation among isolates.
Aim: To identify the spectrum of bacterias associated with wound infection and their antimicrobial susceptibility pattern and biofilm formation.
Introduction: Chronic wounds are severe worldwide problem. Wounds are considered chronic when healing fails to proceed normally and anatomic and functional integrity of the skin is not achieved in approximately 1 month. Chronic wound include diabetic foot ulcer, pressure or decubitus ulcer, venous leg ulcer and non-healing surgical site infection.[1]Annual incidence is of diabetic ulcer in India in a population based study is 1.0 to 4.1% 1. Biofilm formation is widespread in chronic wounds. The biofilm phenotype gives rise to drug resistant strains resulting in treatment failure.
Materials and Methods: A prospective study was done at a tertiary care Hospital in North Karnataka for a period of 1 year from February 2017 to January 2018. A total of 241 samples in an age group of 20 to 70 years with history of wound infection attending surgery department were collected with a sterile cotton swab and processed as per CLSI standards.[2] Detection of biofilm formation by modified congo red agar method, tissue culture plate method and tube assay method.
Results: A total of 241 pus samples were collected. 65.56% (158) showed bacterial growth and 34.43% (83) showed no growth. The study group comprised 139 male and 102 female patients in the ratio of 1.39:1. Age range was 20-70 years Maximum samples were in the age group of 21-30 years. 44.93% (71) of isolates were obtained Gram positive organisms and 55.06 % (87) were Gram negative. Escherichia coli was the predominant isolated organism 16.18% (39). Followed by staphylococcus aureus 9.54% (23). 76 out of 158 sample showed biofilm formation (48.01%). Staphylococcus aureus was dominant biofilm former. 81.57% (62) of biofilm producers were multiple drugs resistant.
Conclusion: Detection of biofilm formation is an easy and cost effective test that can be performed routinely in the lab. Biofilm will help surgeon to effectively manage these infections riding more aggressive source control and appropriate antibiotics resulting in decrease mortality and morbidity of patients.
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Keywords: Surgical wound infection, Biofilm, Antimicrobial resistance.

Introduction

A surgical site wound infection occurs within 30 days after the operation and involves only skin and subcutaneous tissue.[1]

Surgical wound infection is a common cause of nosocomial infections with incidence of 6.1%-38.7%.[2-5] Hospitals with advanced health care facilities also face the problem of surgical site infections even with development in infection control and surgical practices. Factors significantly affecting SSI can be divided into patient associated (senility, preceding infection, nutritional status, concomitant disease), procedure associated (substandard surgical technique, prolonged surgical event, faulty sterilization of equipment, insufficient part preparation before surgery) and microorganism associated (virulence and pathogenicity of bacteria). Organisms causing infection may vary with geographical location, type of procedure being carried out, surgeons operating, between different hospitals and also between wards of same hospital the organisms that colonize the surface wounds provide an ideal niche for invasion in surgical site infection. Common organisms involved in SSI include staphylococcus aureus (37%), pseudomonas aeruginosa (17%), proteus mirabilis (10%), escherichia coli (6%) and corynebacterium spp. (5%).[6]

Aberrant use of broad spectrum antibiotics and developing antimicrobial resistance with indigent infection control practice and overcrowded hospitals have detonated the condition of SSI.

The problem gets more complicated with infections caused by organisms producing biofilm, which is micro communities of organisms within a matrix of extracellular polymeric substance,[7] which is widespread in chronic wounds. These biofilm phenotypes give rise to multidrug resistant strains resulting in treatment failure as the bacteria are protected from various stress like host immune response and antibiotics.

Materials and Methods

A prospective study was conducted from February 2017 to January 2018 in a tertiary care hospital in North Karnataka, India. Approval from IEC was obtained. A total of 241 samples in an age group of 20-70 years with history of wound infection attending surgery department were collected with 2 sterile cotton swabs, a

detailed history regarding age, sex, type of illness, diagnosis, type and duration of surgery performed, antibiotic therapy and associated comorbid disease was obtained from the patient. Provisional diagnosis was made from one swab by Gram stained preparation. The other swab was inoculated on 5% sheep blood agar and MacConkey agar plates and incubated at 37^0C for 24 – 48 hours. Growth on culture plate was identified by its colony characters and the battery of standard biochemical tests.[8] Antimicrobial susceptibility test was done by modified Kirby Bauer disc diffusion method on Mueller Hinton agar and results were interpreted as per CLSI guidelines.[9]

Detection of biofilm formation was done by modified Congo red agar method,[10] Tube assay [11] and tissue culture plate technique.[12]

Congo red agar is a specially prepared medium composed of brain heart infusion broth (37g/L), sucrose (50g/L), agar (10g/L) Congo red stain (0.8g/L). Congo red was prepared as a concentrated aqueous solution and autoclaved at 121^0C for 15 min, separately from other medium constituents and was added when agar had cooled to 55^0C plates were inoculated and incubated aerobically for 24-48 hours at 37^0C biofilm formers produced black colonies with a dry crystalline consistency, while weak slime producers usually remained pink. Indeterminate results were characterized by darkening of colonies with absence of dry crystalline colony morphology. The tests were carried out in triplicate and repeated 3 times.

Fig. 1: Congo red agar method

Tube Assay Methods: A loopful of test organisms was inoculated in 10mL of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at 37^0C for 24–48 hours. After incubation, tubes were decanted and washed with phosphate buffer saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water. Tubes were dried in inverted position. Biofilm formation was considered positive when visible film lined the wall and bottom of tube. The experiment was performed in triplicate and repeated 3 times.
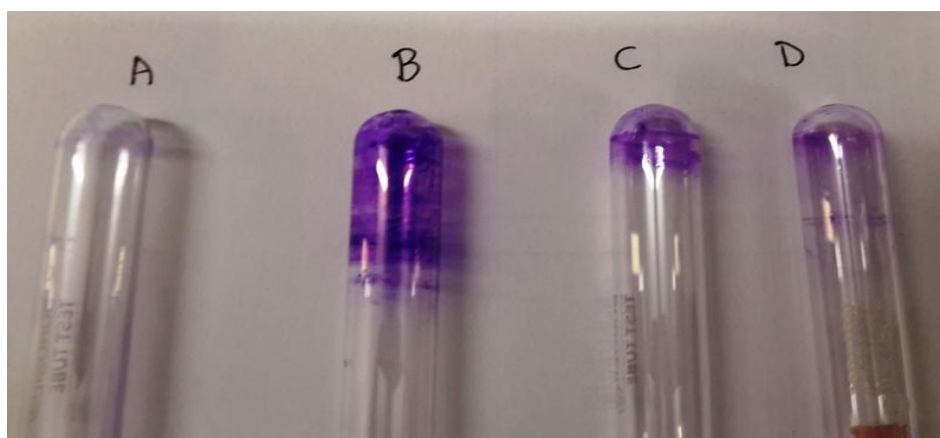
Fig. 2: Tube assay method

Tissue Culture Plate Method: On a sterile 96 well flat-bottomed polystyrene microtiter plate, 230μl of trypticase Soya Broth (TSB) was added. Also, 20μl of overnight bacterial culture was added to the corresponding well (each strain in three successive wells). The negative control wells contained broth only. The plates were incubated aerobically for 24 hours at 35°C. The content of the wells was poured off and the wells were washed three times with 300μl of sterile distilled water. The bacteria adhering to the wells were fixed with 250μl of methanol for 15 minutes. Then the wells were stained with 250μl of 1% crystal violet solution for five minutes. Excess stain was removed by washing and the wells were air-dried. The dye bound to the wells was solubilized with 250μl of 33 per cent (v/v) glacial acetic acid. The optical density (O.D.) of each well was measured at 490nm using an ELISA auto reader.

The tests were carried out in triplicate and the results were averaged. The cut-off O.D (Owas determined as three standard deviations above the mean O.D. of the negative control. Strains were classified as biofilm producer and no biofilm producer. Data was

compiled and descriptive statistics were applied using Microsoft Excel 2010 Edition (Microsoft, Seattle, WA). **Limitations:** anaerobic culture and MIC were not performed in our study.

Results

A total of 241 pus samples were collected. 65.56% (158) showed bacterial growth and 34.43% (83) showed no growth. The study group comprised 139 males 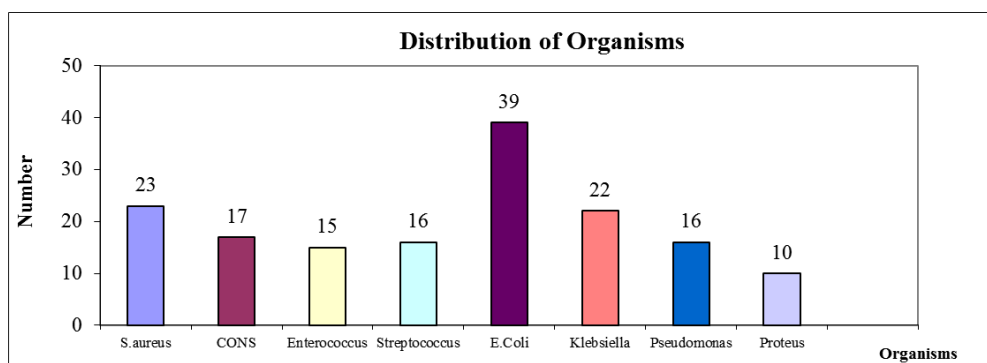and 102 female patients in the ratio of 1.39:1. Maximum samples were in the age group of 21-30 years. 44.93% (71) of isolates were Gram positive organisms and 55.06% (87) were Gram negative.

Escherichia coli was the predominant isolated organism 16.18% (39). Followed by *Staphylococcus aureus* 9.54% (23). 76 out of 158 sample showed biofilm formation (48.01%). *Staphylococcus aureus* was dominant biofilm former. 81.57% (62) of biofilm producers were multiple drug resistant.
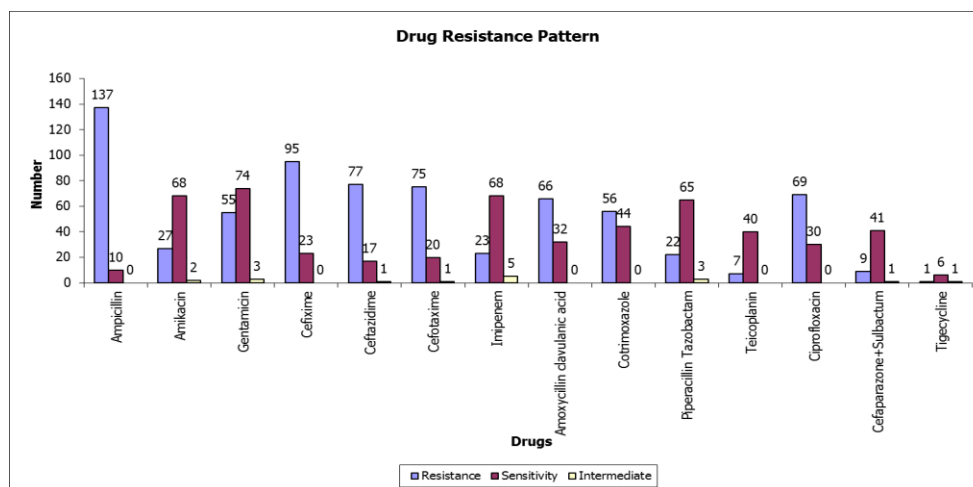
Table 1:

Age group (years)	Male		Female		Total	
	No.	Percent	No.	Percent	No.	Percent
20-30	39	28.05	69	67.64	108	44.81
30-40	27	19.42	16	15.68	43	17.84
40-50	32	23.02	7	6.86	39	16.18
50-60	25	17.98	4	3.92	29	12.03
60-70	16	11.53	6	5.90	22	9.13
	139	100.00	102	100.00	241	100.00

Graph 1:

Graph 2:

Graph 3:

Multiple Drug Resistant Vs Biofilm Former

No biofilm 18%

Biofilm former 82%
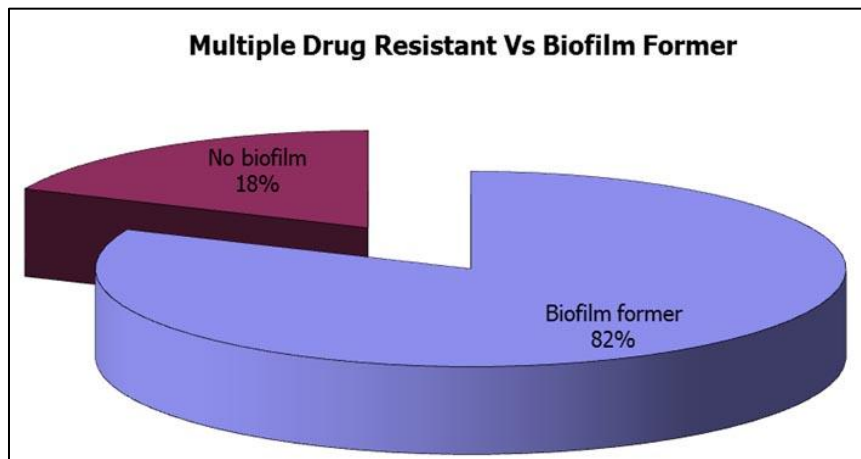
Discussion

A total of 241 samples were processed in our study of which 65.56% showed significant growth. Aynalem et al[13] observed bacterial isolation rate of 83.9 %(115) although male (59.1%) female (40.9%) ratio and majority of infections in an age group of 16-40 years were alike. In a similar study by Basista et al[14] isolated *S. aureus* as predominant isolate followed by pseudomonas. Gram positive were susceptible to cefoxitin, gentamicin & Gram negative to amikacin, ciprofloxacin. Similar to our study Rugira et al[15] observed *E.coli* as predominant (51.2%) multiple drug resistant followed by *S. aureus* (21.1%) resistant to cloxacillin & vancomycin. Pseudomonas was least resistant. Banu et al[16] also observed *S. aureus* was predominant biofilm former (20%). 68.9% multiple drug resistant strains were biofilm former. Zubair et al[17] also observed that 60 (59.4%) of gram negative isolates were biofilm producer with *P vulgaris* (80%) as predominant followed by *K pneumoniae* (77.7%), *E coli* (63.4%), *Acinetobacter* (60%) high degree of antibiotic resistance was exhibited by all biofilm former compared to non biofim former against cefoperazone (79.6%), piperacillin (68.4%), cefotaxime (67.3%), amoxyclav (64.3%) amikacin and gentamicin (40.8% each) imepenem (24.5%).

Conclusion

Successful management of bacteria in a wound is of great importance; however, it is still a complex issue. Detection of biofilm formation is an easy and cost-effective test that can be performed routinely in the lab. Biofilm detection will help surgeon to effectively manage chronic infections and their complication by use of antibiofilm peptides which destroy initial attachment to surface and their formation interfering cell signaling pathway riding more aggressive source control and appropriate antibiotics along with strict infection control measures, maintenance of optimal patient care before during and after operation will

surely result in decreased mortality and morbidity of patients.

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