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Antimicrobial susceptibility pattern of bacterial isolates from surgical infected wounds swab at Rafatullah community hospital Bogra, Bangladesh

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Abstract

Background: Wound infection is one of the major problems in surgical cases that are caused and aggravated by the invasion of pathogenic organisms. Information on local pathogens and sensitivity to antimicrobial agents is crucial for successful treatment of wounds. **Objective:** To identify local pathogens and determine antimicrobial susceptibility pattern of isolated bacterial from wound infection at Rafatullah Community Hospital, Bogra. **Methodology:** This cross sectional study was conducted among patients with wound infection visiting Rafatullah Community Hospital, Bogra, from May2014 to December 2015. Wound swab was collected using sterile cotton swabs and processed for bacterial isolation and susceptibility testing to antimicrobial agents following standard bacteriological techniques. Biochemical tests were done to identify the species of the organisms. Sensitivity testing was done using Kirby- Baur disk diffusion method against 15 classes of antimicrobials. The data was analyzed for descriptive statistics using Microsoft Excel. **Results:** In this study 122 bacterial isolates were recovered from 155 specimens showing an isolation rate of 78.6%. The predominant bacteria isolated from the infected wounds were Staphylococcus aureus 36 (29.5%) followed by Escherichia coli 34 (27.9%), Enterobacter species 24 (19.7%), Klebsiella species 14 (11.5%), Pseudomonas species 10 (8.2%) and Enterococcal species 04(3.3%). All isolates showed high frequency of resistance to amoxycillin, amoxicillin-clavulanic acid, co-trimoxazole, and cephradine. **Conclusion:** On in vitro sensitivity testing, amoxycillin, amoxicillin-clavulanic acid, co-trimoxazole, and cephradine were the least effective. Moxifloxacin, gentamicin, amikacin and ciprofloxacin were the most effective antibiotics for all isolates.

Keywords: Bacterial pathogens, Drug resistance, Wound infection.

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]. Exposure of subcutaneous tissue following a loss of skin integrity (i.e. wound) provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. Since wound colonization is most frequently poly-microbial, involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected[2]. Infection in wound constitutes a major barrier to healing and can have an adverse impact on the patient's quality of life as well as on the healing rate of the wound. Infected wounds are likely to be more painful, hypersensitive and odorous, resulting in increased discomfort and inconvenience for the patient[3]. The prevalent organisms that have been associated with wound infection include Staphylococcus aureus which from various studies have been found to account for 20-40% and Pseudomonas aeruginosa 5-15% of the nosocomial infection, with infection mainly following surgery and burns. Other pathogens such as Enterobacter species, Klebsiella species, Enterococci species are members of the Enterobacteriaceae have been implicated, especially in immune compromised patients and following abdominal surgery[4].

Wound healing needs a good healthy environment so that the normal physiological process will result in a normal healing process with minimal scar formation. One of the most important strategies to keep the process of healing ongoing is to sterilize damaged tissue from any microbial infection[5]. Continued use of systemic and topical antimicrobial agents has provided the selective pressure that has led to the emergence of antibiotic resistant strains which in turn, has driven the continued search for new agents. Unfortunately, the increased costs of searching for effective antimicrobial agents and the decreased rate of new drug discovery has made the situation increasingly worrisome[6]. Hence the present study is designed to update profile of bacteria present in wounds and hospital environment, their sensitivity to antibiotics at Rafatullah community hospital, Bogra, Bangladesh.

Methodology

Study design and Setting

This cross sectional study was conducted at TMSS Medical College & Rafatullah Community Hospital, Bogra, Bangladesh with duration from May 2014 to December 2015.

Sampling procedure

A questionnaire was used to obtain data from the patient after obtaining an informed consent from the patient/guardians. Open and dressed wound swabs were aseptically obtained by sterile cotton swab by rotating with sufficient pressure.

Isolation and identification

Collected swabs were streaked on Hi-chrome, blood agar, and MaCconkey agar and Brain heart infusion broth for enrichment culture by sterile inoculation loop. The plates were incubated at 37°C for 24-48 hours. Preliminary identification of bacteria was based on colony characteristics of the organisms such as haemolysis on blood agar,

changes in physical appearance in differential media and enzyme activities of the organisms. Biochemical tests were performed on colonies from primary cultures for identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests, namely: Triple sugar Iron Agar (TSI), Simon's citrate agar, motility indole urea (MIU). Gram-positive cocci were identified based on their gram reaction, catalase, coagulase test results and subculture on Mannitol salt agar (MSA).

Antibacterial susceptibility testing (AST)

Susceptibility testing was performed by Kirby-Bauer disk diffusion technique according to criteria set by CLSI 2011. The inoculum was prepared by picking parts of similar test organisms with a sterile wire loop and suspended in sterile normal saline. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5 Barium sulphate solution. The test organism was uniformly seeded over the Mueller-Hinton agar surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic-impregnated paper disk into the agar medium, and then incubated at 37°C for 24 hours. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler and classified as sensitive, intermediate, and resistant according to the standardized table supplied by CLSI 2011.

The drugs tested for both gram negative and gram positive bacteria were amoxycillin (10 µg), amoxicillin-clavulanic acid (30 µg), cephradine (25 µg), cefuroxime(30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), moxifloxacin (10 µg), azithromycin(30 µg), amikacin (30 µg), gentamicin (10 µg), cotrimoxazole (25 µg), and meropenem (30 µg). Erythromycin (15 µg) and vancomycin (30 µg) were used for only gram positive bacterial isolates. These antimicrobial selected based on the availability and prescription frequency of these drugs in the study area.

Results

A total of 155 specimens were collected from patients with clinical evidence of wound infection (patients with complaints of discharge, pain, swelling, foul smelling and chronic wound) from May 2014 to December 2015. The subjects included 107 (69%) males and 48 (31%) females. The ages of the patients ranged from 6 years to 90 years with mean age of 42±17.12 (Table 1)

Table 1: Antibiotic susceptibility pattern of gram positive bacteria isolated from patients.

Sex	Infected No. (%)	Not infected No. (%)	Total No. (%)
Male	87 (71.3)	20 (10.3)	107 (71.3)
Female	35 (28.7)	13 (18.6)	48 (28.7)
Total	**122 (78.5)**	**33 (21.5)**	**155 (100)**

Bacterial profile

Of the 155 swabs 122 (78.5%) were culture positive for bacterial pathogens, while 33 (21.5%) were showed no growth. The presence of only one species isolated from each sample was the most frequent (91.6%) while, more than one species were isolated from (8.4%) of the total swabs. A total of 122 bacterial isolates were obtained, 82 (67.2%) were gram negative while 40 (33.8%) were gram positive. Staphylococcus aureus was the predominant organism isolated 36 (29.5%), followed by Escherichia coli 34 (27.9%), Enterobacter species 24 (19.7%), Klebsiella species 14 (11.5%), Pseudomonas species 10 (8.2%) and Enterococcal species 04(3.3%) (Table 2 and Figure 1).

Table 2: Bacterial distribution in isolates

Isolates	Number	Percentages
Staphylococcus aureus	36	29.5
Escherichia coli	34	27.1
Enterobacter species	24	19.7
Klebsiella species	14	11.5
Pseudomonas species	10	8.2
Enterococcal species	04	3.3
Total	122	100

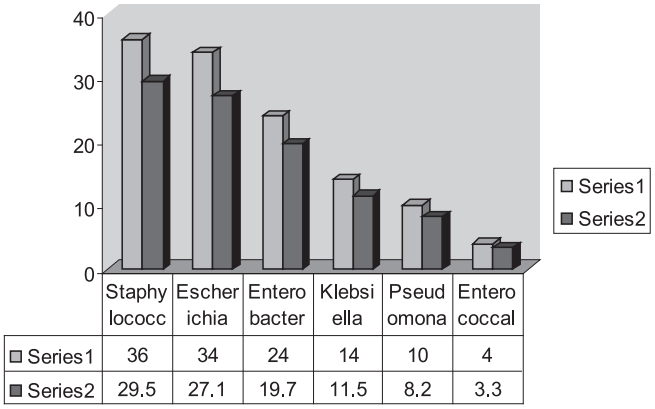

Figure-1: Bacterial distribution in isolates

Antimicrobial susceptibility pattern of bacterial isolates

Gram positive bacteria

Gram positive bacteria were tested against selected 14 antibiotics. The results obtained showed that the organisms varied in their susceptibility to all the antimicrobials used. Majority of them showed multi-resistances (resistance to two or more classes of antimicrobials). Rate of isolates resistant to amoxicillin was 94%, followed by amoxicillin-clavulanic acid (79%), co-trimoxazole (79%) and azithromycin (50%). All isolates were showed sensitive to Meropenem (95%), Moxifloxacin (95%), vancomycin (89%), amikacin (89%) and ciprofloxacin (89%) (Table 3).

Table 3: Antibiotic susceptibility pattern of gram positive bacteria isolated from patients.

Isolate	S/R	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
S.aureus	S	5.6	22.2	66.7	83.3	83.3	72.2	88.9	94.4	50.0	88.9	94.4	22.2	94.4	72.2	88.9
	R	94.4	77.8	33.3	16.7	16.7	27.8	11.1	5.6	50.0	11.1	5.6	77.8	5.6	27.8	11.1
E.cocci	S	00	00	00	00	00	00	50	50	50	00	50	50	100	25	75
	R	100	100	100	100	100	100	50	50	50	100	50	50	00	75	25

KEY: S: Sensitive; R: Resistant; 1. Amoxycillin;
2. Amoxycillin-clavulanic acid; 3. Cephradine; 4. Cefuroxime;
5. Ceftriaxone; 6. Ceftazidime; 7. Ciprofloxacin; 8. Moxifloxacin;
9. Azithromycin; 10. Amikacin; 11. Gentamicin;
12. Trimethoprim-sulphamethoxazole; 13. Meropenem 14. Erythromycin
15. Vancomycin

Gram negative bacteria

The susceptibility patterns of gram negative bacteria (n = 82) isolated from wound infections and tested against selected 13 antimicrobial agents. Rate of isolates resistant to amoxycillin was 88%, followed by amoxicillin-clavulanic acid (77-100%), azithromycin (50-58%), co-trimoxazole (50-65%) and cephradine (47-924% (Table 4).

Table 4: Antibiotic susceptibility pattern of gram negative bacteria isolated from patients.

Isolate	S/R	1	2	3	4	5	6	7	8	9	10	11	12	13
E.coli	S	11.8	23.5	52.9	52.9	52.9	52.9	70.6	70.6	41.2	82.4	82.4	41.2	76.5
	R	88.2	76.5	47.1	47.1	47.1	47.1	29.4	29.4	58.8	17.6	17.6	58.8	23.5
E.bacter	S	8.3	8.3	50.0	75.0	66.6	41.7	83.3	91.7	83.3	91.7	91.7	50.0	100
	R	91.7	91.7	50.0	25.0	33.4	58.3	16.7	8.3	16.7	8.3	8.3	50.0	00
Kleb	S	14.3	14.3	42.8	42.8	14.3	14.3	57.1	71.4	71.4	71.4	42.8	57.1	14.3
	R	85.7	85.7	57.2	57.2	85.7	85.7	42.9	28.6	28.6	28.6	57.2	42.9	85.7
Pseud	S	00	00	00	00	00	00	50.0	50.0	50.0	00	50.0	50.0	100
	R	100	100	100	100	100	100	50.0	50.0	50.0	100	50.0	50.0	00

KEY: S: Sensitive; R: Resistant; 1. Amoxycillin;
2. Amoxycillin-clavulanic acid; 3. Cephradine; 4. Cefuroxime;
5. Ceftriaxone; 6. Ceftazidime; 7. Ciprofloxacin; 8. Moxifloxacin;
9. Azithromycin; 10.Amikacin;11. Gentamicin; 12.
Trimethoprim-sulphamethoxazole; 13. Meropenem

Discussion

The incidence of wound infection was more common in males (71.37%) than in females (28.7%). This is in agreement with studies done in different parts of Ethiopia[7-10] and other countries[11-13]. This might be explained by the fact that traditionally, in Bangladesh mainly males are involved in occupations such as farming, construction works, transportation and industry works where the likely exposure to trauma is common.

In this study, 70.5% of culture positive wounds showed mono-microbial growth, 8.0% showed poly-microbial growth and 21.5% had no bacterial growth. Similarly high percentage of mono-microbial growth was reported in India (86-100%) and Pakistan (98%)[14-17].

In our study, Staphylococcus aureus 36 (29.5%) followed by Escherichia coli 34 (27.9%) were the predominant organisms isolated from wound infections. A number of reports done previously on wound infection from Mymensingh, Bangladesh[31] and different parts of the world indicated that Staphylococcus aureus and Escherichia coli were the most frequent isolates[18-21]. The high prevalence of Staphylococcus aureus infection may be because it is an endogenous source of infection. Infection with this organism may also be due to contamination from the environment e.g. contamination of surgical instruments. With the disruption of natural skin barrier Staphylococcus aureus, which is a common bacterium on surfaces, easily find their way into wounds.

In the determination of the susceptibility of Staphylococcus aureus on fifteen selected antibiotics by disk diffusion technique showed that Staphylococcus aureus tend to be resistant to a wider spectrum of antibiotics. In this studies Staphylococcus aureus was highly resistance to amoxycillin (95%), amoxycillin-clavulanic acid (78%) and Co-trimoxazole (78%). This was consistent with study done in Mymensingh, Bangladesh[32], Ethiopia[19-20] and elsewhere[4,11,14,22]. The same isolate was highly sensitive to moxifloxacin (95%), gentamicin (95%) and amikacin (89%), vancomycin (89%). This finding is in agreement with the work of Bess LJ. *et al.*,

Bibi S. et al., Shamsuzzaman et al., Gelaw A. *et* al., Gautam R *et al.*, and Shriyan A. et al.*,*[7,15,22-25], who reported that clinical *Staphylococci* are 100% sensitive to vancomycin and to amikacin[23,26,27]. Remarkable susceptibility of gram positive bacteria to vancomycin, amikacin and aminoglycosides (gentamicin) may be due to lesser use of these antibiotics as a result of their less availability, cost and toxic effect respectively.

In this study, 88% of the Escherichia coli isolates were resistant to amoxycillin (96.6%), amoxycillin-clavulanic acid (77%), ceftriaxone (62%), Azithromycin (59%) and sulphamethoxazole trimethoprim (55%). Sensitivity pattern of Escherichia coli in our study as compared to others were ciprofloxacin (65.5%), moxifloxacin (71%) and amikacin(82%)[8,19,27,28]. So, reduced antibiotic sensitivity pattern noted for Escherichia coli suggests its importance for hospital acquired infection.

Klebsiella species was 86% resistance to amoxycillin, sulphamethoxazole trimethoprim and cephradin, (71%) in ceftriaxone however it indicates low resistance to ciprofloxacin (35.7%). This was in consistence with the study done in Ethiopia[7,8,20,29]. Most of the gram negative bacteria isolated were resistant to amoycillin, amoxycillin-clavulanic acid, cephradine and ceftriaxone. This may be due to the antibiotics having been in use for much longer time and their oral route of administration that affects their rate of absorption into blood stream. Some of them were used as prophylaxis therefore increasing their use in patients. Over use of antibiotics contributes to organisms developing resistance.

In our study Pseudomonas species showed reduced sensitivity to commonly used antibiotics like amoxycillin, amoxycillin-clavulanic acid, cephradine, ceftriaxone and ceftazidime. Meropenem, moxifloxacin, ciprofloxacin and gentamicin has been stated to be the most potent drug available for the treatment of Pseudomonas species infections. This report is in conformity with the result of other study in which ciprofloxacin recorded the least resistance (6.2-24%) to Pseudomonas species isolates from wound infection[20,28,30]. It is undoubtable that at the present time, the oral drug ciprofloxacin and injection gentamicin are the most effective antibiotics against Pseudomonas species involved in wound infection relative to most other commonly used drugs. Pseudomonas resistant to third generation cephalosporins (ceftriaxone 63.6%) is real treat. In fact, the irrational and inappropriate use of antibiotics is responsible for the development of resistance of Pseudomonas to antibiotic monotherapy. The incidence of Pseudomonas species in wound infection among admitted patient is becoming more serious in developing countries because of lack of general hygienic conditions, production of low quality antiseptics and medicinal solutions for treatment[11].

Conclusion

The most common isolate in wound infection was Staphylococcus aureus followed by Escherichia coli, Enterobacter species, Klebsiella species, Pseudomonas species, and Enterococcal species. These isolates showed high frequency of resistance to amoxycillin, cephradine, amoxicillin-clavulanic acid and Trimethoprim-sulphamethoxazole. Thirty three and sixty seven percent of Gram positive and Gram negative isolates respectively.

Competing interests

The authors declare that they have no competing interests.

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