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Original Article

Antimicrobial Susceptibility Pattern of Bacterial Isolates from Wound Infection

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Abstract

Objective: The present study was done to see the antimicrobial susceptibility pattern of bacterial isolates from wound infection.

Methods: Wound swabs collected from 150 patients of wound infection were cultured and microbial isolates identified using standard methods. Antimicrobial susceptibility testing was done on bacterial isolates by Modified Kirby Bauer method.

Results: Of the 150 swabs 131 (87.4%) were culture positive for bacterial pathogens, while 19 (12.6%) were bacteriologically sterile showing an isolation rate of 87.4%. The predominant bacteria isolated from the infected wounds were *Staphylococcus aureus* 47 (32.4%) followed by *Escherichia coli* 29 (20%), *Proteus species* 23 (16%), Coagulase negative *Staphylococci* 21 (14.5%), *Klebsiella pneumoniae* 14 (10%) and *Pseudomonas aeruginosa* 11 (8%).In case of gram positive bacteria- rate of isolates resistant to ampicillin was 94%, followed by penicillin G, 86.8%, Tetracycline, 51.5%. In case of gram negative bacteria- rate of isolates resistant to ampicillin was 96%, followed by cephalothin, 92.4%, Tetracycline, 74%.

Conclusion: In antimicrobial susceptibility testing ampicillin, penicillin, cephalothin and tetracycline were the least effective. Gentamicin, norfloxacin, ciprofloxacin, vancomycin and amikacin were the most effective antibiotics.

Keywords: Antimicrobial susceptibility testing, bacterial isolates, wound infection, antibiotic resistances.

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Introduction

The intact skin serves to control microbial organism that live on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens[1]. Loss of skin integrity 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].

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The commonest organisms 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 E coli, Enterococci and other members of the Enterobacteriaceae have been implicated 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].

Most hospitals in developing countries especially Bangladesh, have rudimentary and highly compromised infection control programmes due to lack of awareness of the problem, lack of personnel, poor water supply, erratic electricity supply, poor laboratory back up and funding. These factors are common in most rural health care centers in Bangladesh. Accurate information of the incidence and etiology of infections acquired within a hospital is essential for effective preventive measures. Against this background, this study was aimed at determining the prevalence of wound infection and susceptibility profile of associated aerobic bacteria from patients at a rural tertiary health care facility in Bangladesh.

Methods

Patients

A total of 150 specimens were collected from patients with clinical evidence of wound infection (patients with complaints of discharge, pain, swelling, foul smelling and chronic wound) from November, 13 to October, 14 at Microbiology Department of RMCH.

A pair of wound swab was collected from each patient. One of the wound swabs was used to make film and stained by gram's stain. The second swab was cultured onto blood, MacConkey agar and incubated for 24 to 48 hours at 37°C. Bacterial isolates were identified using standard laboratory techniques[7,8]. Antimicrobial susceptibility test for bacterial isolates was performed using the modified Kirby –Bauer method.

The drugs tested for both gram negative and gram positive bacteria were ampicillin (10 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), cephalothin (30 µg), gentamicin (10 µg), tetracycline (30 µg), cotrimoxazole (25 µg), chloramphenicol (30 µg), doxycycline (30 µg), naldixic acid (15 µg) and ceftriaxone (30 µg). Penicillin G (10 IU), erythromycin (15 µg) and vancomycin (30 µg) were used in addition only for gram positive bacterial isolates (oxoid). These antimicrobial selected based on the availability and prescription frequency of these drugs in the study area.

Results

A total of 150 specimens were collected from patients with clinical evidence of wound infection from November, 13 to October, 14 at RMCH. The subjects included 107 (71.3%) males and 43 (28.7%) females. The ages of the patients ranged from 6 months to 90 years.

Of the 150 swabs 131 (87.4%) were culture positive for bacterial pathogens, while 19 (12.6%) were bacteriologically sterile. 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 145 bacterial isolates were obtained, 77 (53%) were gram negative while 68 (47%) were gram positive. *S. aureus* was the predominant organism isolated 47 (32.4%), followed by *Escherichia coli (E. coli)* 29 (20%), *Proteus* spps 23 (16%), coagulase negative *Staphylococci* (CONS) 21 (14.5%), *Klebsiella pneumoniae (K. pneumoniae)* 14 (10%) and *P. aeruginosa* 11 (8%) **(Table -1).**

Table -1 : Pattern of bacteria isolated from wound swab (N=145)

Bacteria	Number	Percentage
Staph. aureus	47	32.4
E coli	29	20
Proteus spp	23	16
CONS	21	14.5
K pneumoniae	14	10
P aeruginosa	11	8
Total	145	100

Percentage of bacteria isolated from patients with infected wounds

Antimicrobial susceptibility pattern of bacterial isolates

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 ampicillin was 94%, followed by penicillin G, 86.8%. All isolates were 100% susceptible to vancomycin and amikacin, and showed low resistance to norfloxacin (10%), ciprofloxacin (10%), sulphamethoxazole trimethoprim (8.8%) and gentamicin (8.8%) (Table-2).

Table 2 : Antibiotic susceptibility pattern of gram positive bacteria isolated from patients

Isolates		Antimicrobial agents (%)													
		CN	VA	AK	E	C	SXT	NOR	P	KF	CRO	TE	CIP	AP	DO
S.aures (n = 47)	S	45 (96)	47 (100)	47 (100)	40 (85.1)	40 (85.1)	44 (94)	45 (96)	4 (8.5)	33 (70.2)	40 (85.1)	23 (49)	45 (96)	2 (4.3)	34 (72.4)
	R	2 (4)	-	-	7 (14.9)	7 (14.9)	3 (6)	2 (4)	43 (91.5)	14 (29.8)	7 (14.9)	24 (51)	2 (4)	45 (95.7)	13 (27.6)
CONS (n = 21)	S	17 (81)	21 (100)	21 (100)	13 (62)	14 (67)	18 (86)	16 (76.2)	5 (24)	6 (29)	15 (71.4)	10 (48)	16 (76.2)	2 (9.5)	15 (71.4)
	R	4 (19)	-	-	8 (38)	7 (33)	3 (14)	5 (23.8)	16 (76)	15 (71)	6 (28.6)	11 (52)	5 (23.8)	19 (90.5)	6 (28.6)
Total (n = 68)	**S**	**62 (91.2)**	**68 (100)**	**68 (100)**	**53 (78)**	**54 (79.4)**	**62 (91.2)**	**61 (90)**	**9 (13.2)**	**39 (57.4)**	**55 (81)**	**33 (48.5)**	**61 (90)**	**4 (6)**	**49 (72.1)**
	R	**6 (8.8)**	-	-	**15 (22)**	**14 (20.6)**	**6 (8.8)**	**7 (10)**	**59 (86.8)**	**29 (42.6)**	**13 (19)**	**35 (51.5)**	**7 (10)**	**64 (94)**	**19 (27.9)**

KEY: S: Sensitive; R: Resistant; −: zero; CN: Gentamicin; V: Vancomycin; AK: Amikacin; E: Erythromycin; C: Chloramphenicol; SXT: Trimethoprim-sulphamethoxazole; NOR: Norfloxacin; P: Penicillin; KF: Cephalothin; CRO: ceftriaxone; TE: Tetracycline; CIP: Ciprofloxacin AP: Ampicillin; DO: Doxycycline.

The susceptibility patterns of gram negative bacteria (n = 77) isolated from wound infections and tested against selected 11 antimicrobial agents. Rate of isolates resistant to ampicillin was 96%, followed by cephalothin, 92.4% (Table-3).

Table -3 : Antibiotic susceptibility pattern of gram negative bacteria isolated-

Isolates		Antimicrobial agents (%)										
		CN	C	SXT	NA	NOR	KF	CRO	TE	CIP	AP	DO
E.coli (n = 29)	**S**	14 (48.3)	10 (34.5)	13 (45)	17 (59)	16 (55.2)	0	11 (38)	6 (21)	19 (66)	-	16 (55.2)
	R	15 (51.7)	19 (65.5)	16 (55)	12 (41)	13 (44.8)	29 (100)	18 (62)	23 (79)	10 (34)	29 (100)	13 (44.8)
Proteus Spp (n = 23)	**S**	17 (74)	16 (70)	14 (61)	15 (65.2)	20 (87)	3 (13)	8 (35)	6 (26)	19 (83)	2 (9)	13 (57)
	R	6 (26)	7 (30)	9 (39)	8 (34.8)	3 (13)	20 (87)	15 (65)	17 (74)	4 (17)	21 (91)	10 (43)
K. pneumoniae (n = 14)	**S**	5 (36)	2 (14.3)	2 (14.3)	7 (50)	11 (79)	2 (14.3)	4 (29)	6 (43)	9 (64.3)	-	8 (57.1)
	R	9 (64)	12 (85.7)	12 (85.7)	7 (50)	3 (21)	12 (85.7)	10 (71)	8 (57)	5 (35.7)	14 (100)	6 (42.9)
P. aeruginosa (n = 11)	**S**	9 (82)	2 (18.2)	3 (27.3)	-	11 (100)	Nt	4 (36.4)	2 (18.2)	11 (100)	-	-
	R	2 (18)	9 (82)	8 (73)	11 (100)	-		7 (63.6)	9 (82)	-	11 (100)	11 (100)
Total (n = 77)	**S**	**45 (58.4)**	**30 (39)**	**32 (42)**	**39 (51)**	**58 (75.3)**	**5 (7.6)**	**27 (35.1)**	**20 (26)**	**58 (75.3)**	**3 (4)**	**37 (48.1)**
	R	**32 (41.6)**	**47 (61)**	**45 (58)**	**38 (49)**	**19 (24.7)**	**61 (92.4)**	**50 (64.9)**	**57 (74)**	**19 (24.7)**	**74 (96)**	**40 (51.9)**

KEY: S = Sensitive R = Resistant; −: zero; Nt: Not tested; CN: Gentamicin; C: Chloramphenicol; SXT: Trimethoprim-sulphamethoxazole; NOR: Norfloxacin; KF: Cephalothin; CRO: ceftriaxone; TE: Tetracycline; CIP: Ciprofloxacin AP: Ampicillin; DO: Doxycycline.

Discussion

The incidence of wound infection was more common in males (89.7%) than in females (81.4%). This is in agreement with studies done in different parts of Bangladesh[-----] and other countries[9,10, 11]. This might be explained by the fact that traditionally, in this country 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, 91.6% of culture positive wounds showed mono-microbial growth, 8.4% showed poly-microbial growth and 12.7% had no bacterial

growth. Similarly high percentage of mono-microbial growth was reported in India (86-100%) and Pakistan (98%)[12,13].

In our study, *S. aureus* (32.4%) and *E. coli* (20%) were the predominant organisms isolated from wound infections. A number of reports done previously on wound infection from different parts of the world indicated that *S. aureus* and *E. coli* were the most frequent isolates[14,15,16] . The high prevalence of *S. 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 *S.aureus*, which is a common bacterium on surfaces, easily find their way into wounds.

Coagulase Negative *Staphylococci* accounted for 14.5% of the organisms isolated from wounds in this study. This is not unexpected since the organism is a commensal or normal flora on the skin. Several investigations have reported these organisms as common contaminants of wounds[16].

Resistance to the selected antimicrobials was very high. The average resistance of the isolates to all the antibiotics in gram positive cocci was (99%) and gram negative bacilli (100%). This is similar to the study done in Bangladesh with average resistance of gram positive cocci isolates (100%) and gram negative bacilli isolates (95.5%) respectively[16]. The overall multiple drug resistance (two and above antimicrobial classes) of the isolates in this study was 85% which was in line with previous study done in different parts of the world[15]. High resistance of the isolates to antibiotics may be due to practicing self medication, lack of diagnostic laboratory services or unavailability of guideline regarding the selection of drugs thereby which lead to inappropriate use of antibiotics.

In the determination of the susceptibility of *S. aureus* on fifteen selected antibiotics by disc diffusion technique showed that *S. aureus* tend to be resistant to a wider spectrum of antibiotics. In this studies *S.aureus* was highly resistance to ampicillin (95.7%), penicillin (91.5%) and tetracycline (51%). The same isolate was highly sensitive to amikacin (100%), vancomycin (100%), ciprofloxacin (96%), norfloxacin (96%) and gentamicin (96%). This finding is in agreement with the work of Bess LJ. *et al.*, Bibi S. *et al.*, Shamsuzzaman *et al.*, Gelaw A. *et al.*,[13,17,18] who reported that clinical *Staphylococci* are 100% sensitive to vancomycin and to amikacin[18]. In this study, coagulase negative *Staphylococci* were 100% sensitive to amikacin and vancomycin, sulphamethoxazole trimethoprim (86%), gentamicin (83%) and ciprofloxacin (76.2%). This finding was comparable with the previous studies done in different parts of the world[9]. The same organism was remarkably resistance to ampicillin (90.5%), penicillin (76%), cephalothin (71%) and tetracycline (52%). This finding was comparable with study done in the same country[16] and in other parts of the world[9,12] .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, 100% of the *E.coli* isolates were resistant to cephalothin, ampicillin (96.6%), tetracycline (79%), chloramphenicol (65.5%), ceftriaxone (62%), sulphamethoxazole trimethoprim (55%) and gentamicin (51.7%). Sensitivity pattern of *E.coli* in our study as compared to others were ciprofloxacin (65.5%) and naldixic acid[15]. So, reduced antibiotic sensitivity pattern noted for *E. coli* suggests its importance for hospital acquired infection.

K. pneumoniae was 100% resistance to ampicillin, 85.7% in chloramphenicol, sulphamethoxazole trimethoprim and cephalothin, (71%) in ceftriaxone however it indicates low resistance to ciprofloxacin (35.7%) and doxycycline. This was in consistence with the study done in Bangladesh[16]. *Proteus* species were resistance to ampicillin (91%), cephalothin (87%), tetracycline (73.9%) and ceftriaxone (65%). The isolates were sensitive to ciprofloxacin (83%) and gentamicin (74%). Most of the gram negative bacteria isolated were resistant to ampicillin, cephalothin, tetracycline and chloramphenicol. 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.

In this study *P. aeruginosa* showed reduced sensitivity to commonly used antibiotics like ampicillin, doxycycline, naldixic acid, and tetracycline, except ciprofloxacin, norfloxacin (100%), and gentamicin (82%). This report is in conformity with the result of other study in which ciprofloxacin recorded the least resistance (6.2-24%) to *P. aeruginosa* isolates from wound infection[18]. It is undoubtable that at the present time, the oral drug ciprofloxacin and injection gentamicin are the most effective antibiotics against *P. aeruginosa* 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 *P. aeruginosa* 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[9]. Over use of antibiotics contributes to organisms developing resistance. Prescription of antibiotics without laboratory guidance, cheap to procure, the ease of accessibility encourages their misuse and overuse, leading to the development of bacterial resistance over time. Selective pressure due to repeated use of disinfectants in hospital settings may account for this observation.

Conclusion

The most common isolate in wound infection was *S. aureus* followed by *E. coli*, *Proteus* species, *CONS, K. Pneumoniae and Pseudomonas spp.* These isolates showed high frequency of resistance to ampicillin, penicillin, cephalothin and tetracycline. Seventy one percent (71%) and 97.4% of Gram positive and Gram negative isolates showed MDR respectively with overall MDR of 85%.

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