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Pathogenic Enterobacteria associated with Wound Infections and End-stage Renal Failure

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Abstract

Background: Wounds are contaminated by the presence of non-replicating organisms; and usually all chronic wounds become contaminated, and contaminants may come from the endogenous microflora and/or the environment. Most contaminating organisms are not able to multiply in wounds, e.g. soil microorganisms[1].
Wounds are colonized by replicating micro-organisms adherent to the wound in the absence of injury to the host. Wound healing occurs in the presence of bacteria. Certain bacteria appear to aid wound healing. It is not the presence of organisms but their interaction with the patient that determines their influence on wound healing[2].
Objective: To characterize pathogenic enterobacteria associated with wound infections and end-stage renal failure
Materials and methods: Aspirate or pus was collected using sterile, disposable syringe or a cotton-wool swab. A clean catch mid-stream, urine (MSU) samples were collected in sterile screw-capped, leak-proof, disposable, plastic containers. Wound swabs were transported in Amies transport medium. Urine samples were transported to the laboratory within one hour. The collected wound specimens were inoculated onto blood agar and Mac Conkey agar. Standard bacteriological methods were employed for isolation and identification. Kirby-Bauer technique was used to test the antimicrobial sensitivity of the isolated organisms. The antibiotics used were amoxycillin/clavulanic acid (amoxyclav), cefepime, ciprofloxacin, gentamicin, methicillin, tetracycline, co-trimoxazole, and norfloxacin.
Results: A total of 57 patients were investigated. 32 patients with wound infections and 25 patients with end-stage renal failure. *Escherichia coli* was the predominant (24 %) enterobacteria isolated from end-stage renal failure cases; while *Klebsiella pneumoniae* (21.8 %) was the commonest enterobacteria isolated from wound infection patients. The isolated pathogens were more sensitive to norfloxacin in end-stage renal failure cases, while gentamicin was the best drug for wound infection patients.
Conclusion: Bacterial culture was the most practical method for management of end-stage renal failure and wound infections. Determination of the sensitivity pattern was the most effective procedure to establish proper therapy of wound and urinary tract infections.

Key words: Pathogenic enterobacteria, Wound infections, End-stage renal failure

Introduction

The replicating micro-organisms are normal skin flora, e.g. coagulase negative staphylococci, *Corynebacterium* species, *Brevibacterium* species, *Proprionibacterium acnes*, and *Pityrosporum* species. Pathogens of concern are *Staphylococcus aureus,* β-hemolytic streptococci *(S. pyogenes, S. agalactiae), E. coli, Proteus, Klebsiella,* anaerobes, *Pseudomonas, Acinetobacter,* and *Stenotrophomonas (Xanthomonas).* The microbial flora in wounds appears to change over time. In early acute wound infection, normal skin flora predominate, e.g. *S. aureus*, and then soon followed by beta-hemolytic streptococci (Group B *Streptococcus*) and *S. aureus.* Such organisms are commonly found in diabetic foot ulcers [3].

Urinary tract infection (UTI) is a serious health problem affecting millions of people every year. The annual incidence of chronic renal failure in Sudan is estimated to be about 70-140 per million populations per year. UTI is a condition where one or more structures of the urinary tract become infected. Chronic pyelonephritis is responsible for about 20% of cases of chronic renal failure (CRF) among adults in developed countries, and for up to 29 % in Africa. Patients of CRF are more susceptible to UTI because of altered immunity, uremia, low urinary flow rate and urinary concentration defects. All these factors favor cross multiplication of bacteria. UTI can lead to more deterioration of renal function in patients[4].

UTI can cause serious and permanent renal damage in patients with underlying urinary tract abnormalities, diabetes mellitus, pregnancy, immunocompromised or sickle cell disease. In addition, UTI is a leading cause of gram negative sepsis in hospitalized patients and are the origin of about half of all nosocomial infections caused by urinary catheter[5] .

Urinary tract infections (UTI) and wound infections are devastating problems all over the world and their social and economic effects are well established, and frequently encountered in Sudan. Patients suffer much from these conditions in which bacteria form some of their numerous aetiological agents. Bacterial infections of the urinary tract are commonly seen among outpatients and hospitalized patients. They are often asymptomatic, or symptoms are insufficient to draw attention[6]. Bacterial species play an important role in surgical sepsis. The infection may be endogenous or exogenous, and due to resistant, virulent microorganisms. Different bacterial species were incriminated in the aetiology of wound infections. The significance of these organisms was a dispute among many workers who handled this subject. In Sudan and other similar countries, little work was published on this matter[7].

Bacteria causing UTI and wound infections are usually resistant to the commonly used antibiotics and chemo-therapeutic agents. Adequate treatment and control of these conditions needs a good knowledge of the bacterial species involved and their susceptibility to antimicrobial agents[8].

The object of this study was to determine the Gram negative enterobacteria strains related to UTI and wound infections, and to discuss their place in the causation of these problems. The study also aims to study the susceptibility pattern of these organisms to antibiotics normally applied for treatment of such infections. Such a study would enable clinicians to manage their patients properly, and to choose the best drug for the control of these conditions.

Materials and methods

This was a quantitative, descriptive, facility base study, conducted in the Surgical Department of Khartoum Teaching Hospital and the Renal Dialysis Center (Khartoum, Sudan). The study was carried out during the period from April to July 2009. Approval to run the study was taken from Al Neelain University (Khartoum); and permission to collect the clinical specimens was granted by the authorities of Khartoum Teaching Hospital and Khartoum Renal Dialysis Center. Verbal consent was obtained from all patients enrolled in the study. Data was analyzed by the Statistical Package for Social Science (SPSS) program.

The sample size was 57 patients divided into two groups: 32 patients with wound infections attending Khartoum Teaching Hospital; and 25 patients with end-stage renal failure attending Khartoum Renal Dialysis Center. Sampling selected was a non-probability, convenience type. A structured-interviewing questionnaire was designed to collect demographical and clinical data. All specimens were collected using sterile techniques. Aspirate or pus was collected from wounds sterile, disposable syringe or a cotton-wool swab. Using sterile blades, tissue biopsies were collected.

Clean catch mid-stream, urine specimens were collected in sterile screw-capped, leak-proof, disposable, plastic containers. Urinary catheter was used to collect bladder urine with less urethral contamination. When urine is difficult to obtain, a suprapubic bladder aspiration was made. Specimens were labeled and delivered to the laboratory as soon as possible. Wound swabs were transported to the laboratory in Amies transport medium within one hour. If there was a delay, urine specimens were refrigerated at 4ºC.

The collected wound specimens were inoculated onto plates of 5 % sheep blood agar media and Mac Conkey agar media by using a sterile wire loop. These plates were incubated aerobically at 37ºC overnight. Sheep blood agar and Mac Conkey agar allow the growth of most gram- negative bacilli, staphylococci, streptococci and enterococci. Urine samples were inoculated on cysteine, lactose, electrolyte-deficient (CLED) agar, sheep blood agar, and Mac Conkey agar and incubated aerobically overnight at 37º C.

The colonial morphology was observed and a Gram stain was made to identify the isolates. Colonies were morphologically examined for size, color, haemolysis, and lactose fermentation. The Gram stain reaction of each isolate was determined. Identification of gram positive cocci was made by catalase test, coagulase test, DNase test, and fermentation of mannitol. Identification of *Enterobacteriaceae* was made by Kligler iron agar test, indole production test, citrate utilization test, urease production test, motility test, and oxidase test.

Antimicrobial susceptibility testing of the isolated organisms was performed by the Kirby-Bauer technique. The turbidity of the bacterial suspension was compared to the Mc Farland turbidity standard. The isolates were streaked on Muller-Hinton agar.

The antimicrobial discs used to test the susceptibility of wound infections isolates were amoxycillin, cefepime, ciprofloxacin, gentamicin, methicillin, and tetracycline. While the antibiotics used to test the susceptibility of urinary tract infections isolates were ciprofloxacin, co-trimoxazole, norfloxacin and cefepime. After overnight incubation, the control and test plates were examined to ensure that growth was confluent. Then the diameter of each zone of inhibition was measured in mm. and compared with the Clinical Laboratory Standards Table to determine the organism's susceptibility.

Results

A total of 57 patients were investigated: 32 patients with wound infections and 25 patients with end-stage renal failure.

Regarding wound infections, patients of both genders were investigated. 24 (75%) of them were males and 8 (25 %) were females. The frequency rate of wound infections was highest (65.6%) in the age range 41 to 60 years; and lowest (3.1%) among patients under 20 years. From the 32 wound infection specimens, 20 (62.5 %) pathogenic enterobacteria were isolated (Table I). *Klebsiella pneumoniae* was the predominant (21.8%), while *Proteus vulgaris* was the least pathogenic organism (3.1%) identified.

As regard end-stage renal failure patients, the number of cases investigated were 18 (72%) males and 7(28 %) females. The number of end-stage renal failure cases was highest (44%) in the age range 20 to 30 years; and lowest (24%) among patients in the age range 31-40 years. From these patients 13 (52.5 %) pathogenic enterobacteria were isolated. The organisms causing bacteriuria are listed in Table I. *Escherichia coli* was the predominant (24.0%), while *Proteus* and Providencia spp. were the least organisms isolated (4.0%).

Table I. Enterobacteria isolated from wound infections and end-stage renal failure patients

Enterobacteria isolates		Renal Failure isolates	
Organism	Positive	Organism	Positive
Klebsiella pneumoniae	7(21.8%)	*Escherichia coli*	6(24%)
Escherichia coli	4(12.5%)	*Klebsiella pneumoniae*	5(20%)
Proteus mirabilis	3(9.4%)	*Proteus mirabilis*	1(4%)
Pseudomonas aeruginosa	3(9.4%)	*Providencia* species	1(4%)
Proteus vulgaris	1(3.1%)	Total	13(52%)
Providencia species	2(6.3%)		
Total	20(62.5%)		

On the other hand, sensitivity pattern to antimicrobial agents revealed that enterobacteria isolated from wound infection cases were more sensitive to gentamicin. *Klebsiella pneumoniae* was observed to be highly sensitive to gentamicin, ciprofloxacin, and amoxiclav; while other enterobacteria isolated varied in their sensitivity pattern, ranging from gentamycin and amoxiclav at one end to cefepime and methicillin at the other end (Table II).

Table II: Sensitivity pattern of enterobacteria isolated from wound infections cases

Bacteria isolated	Number of organisms sensitive to:					
	A	T	CF	CP	G	M
Klebsiella pneumoniae	2	1	0	3	4	0
Escherichia coli	3	0	0	0	2	1
Proteus mirabilis	2	1	0	2	3	0
Pseudomonas aeruginosa	1	1	1	0	2	0
Proteus vulgaris	0	0	0	0	1	0
Providencia species	2	0	0	1	2	0
Total	10	3	1	6	14	1

A = Amoxiclav T = Tetracycline CF = Cefepime CP = Ciprofloxacin
G = Gentamycin M = Methicillin

Susceptibility to antimicrobial agents showed that enterobacteria isolated from end-stage renal failure cases were more sensitive to norfloxacin and cefepime (Table III), and *Escherichia coli* was observed to be highly sensitive to these two antimicrobial agents.

Discussion:

In the present study wound infections were more common in males (75%) than in females (25 %). This result agrees with the study performed by other workers[9] who reported a significant risk of wound infections among males.
Also in this study *Klebsiella pneumoniae* was the most frequently isolated (21.8 %) organism in wound infection patients, followed by *E.coli* (12.5 %) and then *Proteus mirabilis* (9.4 %) as demonstrated in Table I. This result is similar to the study of Cutting and his colleagues[10] who found a high incidence of enterobacteria among wound infection cases.
Furthermore, in this context wound infections were more frequent in the age range 41 to 60 years. This agrees with the report of Heinzelmann and his co-workers[2] which revealed that wound infections were also more prevalent in this age group. Bacterial species isolated from wound infection cases in this study (Table I) are similar to those reported by Cutting and his

colleagues[10] who enrolled *Enterobacteriaceae, Pseudomonas aeruginosa,* and *Enterococcus faecalis* in their study.

Operations on the gastrointestinal tract usually carry a potentially high risk of wound infection[11]. This makes enterobacteria isolated in this study a major, endogenous risk factor in maintaining post-operative gastrointestinal infections.

Table III: Sensitivity pattern of enterobacteria isolated from end-stage renal failure patients

Bacteria isolated	Number of organisms sensitive to:					
	A	SXT	CF	CP	G	NOR
Escherichia coli	2	2	5	4	2	5
Klebsiella pneumoniae	1	3	4	4	3	4
Proteus mirabilis	1	1	1	1	1	1
Providencia species	0	1	1	1	0	1
Total	4	7	11	10	6	11

A = Amoxiclav SXT = Co-trimoxazole CF = Cefepime
CP = Ciprofloxacin G = Gentamycin NOR = Norfloxacin

Omer and Suliman[12] in Sudan reported that *Pseudomonas aeruginosa, Proteus mirabilis,* and other members of the *Enterobacteriaceae* family were common when studying the bacteriology of appendicitis. Similar bacterial flora was isolated in our study (Table I).

The choice of antimicrobial agents depends upon the organisms that are likely to colonize the site of infection and capable of providing predictable inhibition of the bacterial growth with the lowest risk of side effects[11]. Enterobacteria isolated among wound infection patients were more susceptible to gentamicin (Table II). This antibiotic is relatively safe when administered under close supervision of the clinician.

Urinary tract infection is one of the most common causes of end-stage renal failure. In addition, patients with chronic renal failure are more prone to develop urinary tract infection[13]. In the present study the frequency rate of bacteriuria among end-stage renal failure patients was 52.0% (Table I). This result was slightly higher than that detected by Foxman[14] who found a frequency rate of 27% of bacteriuria among haemodialysis patients. The current study finding may be explained by the vast deterioration of renal function and the increasing frequency of stone formation in the patients studied.

A single mid-stream urine culture appeared highly reliable in the diagnosis of urinary tract infection[15]. On the other hand, the importance of enterobacteria in urinary tract infections has long been known in many countries[16]. In Sudan, the frequency rate of bacteriuria among UTI patients was studied by Omer and El-Haj[15] who detected a frequency rate of 45% among the cases investigated. In our study, out of the 13 isolates detected among end-stage renal failure patients six (24%) were *E. coli*, followed by five isolates of *Klebsiella pneumoniae* (20 %) as shown in Table I. This supports the study of Foxman and his colleagues[13] who found that the commonest cause of UTI in end-stage renal failure patients was *E.coli*. Also, Omer and El-Haj[15] in their study in Sudan found that the commonest organism isolated was *E. coli* (45%).

Of 163 subjects suffering from urinary tract problems investigated by El- Haj and Chugh[17], 51.8% were females and 48.2% were males. In our study the end-stage renal failure patients were 18 (72%) males and 7 (28 %) females. The number of end-stage renal failure cases was higher in the age range 20 to 30 years. This may be explained by the high frequency of urinary and genital infections among young males and females in this active sexual age group.
Some workers found that nitrofurantoin and nalidixic acid to be the best antimicrobial agents[14] [28]. In this study norfloxacin and cefepime were shown to be the most potent for treatment of UTI in patients with end-stage renal failure (Table III).
From this study it may be recommended that wound infection patients should be early investigated and antibiotic sensitivity pattern must be fully determined to provide effective therapy. Health education programs should be organized by health authorities to reduce the risks of UTI and end-stage renal failure. Thorough laboratory investigations should be directed towards patients with chronic renal failure and UTI to favor prompt diagnosis and treatment.
Conclusion: Bacterial culture was the most practical and sensitive procedure for the diagnosis of UTI, end-stage renal failure, and wound infections. Determination of the sensitivity pattern of pathogenic organisms was the shortest way and the most effective procedure to establish proper therapy of wound infections and UTIs.

References

1. Calvin M. Cutaneous wound repair. *Wounds* 1998; 10(1): 12-32.
2. Heinzelmann M, Scott M, Lam T. Factors predisposing to bacterial invasion and infection. *Am J Surg* 2002; 183(2): 179-90.
3. Plowman R. The socioeconomic burden of hospital acquired infection. *Euro Surveill* 2000; 5(4): 49-50.
4. Bibbings J. Honey, lizard dung and pigeons' blood. *Nurs Times* 1984; 80(48): 36-38.
5. Ellis T. Treating the wounded: Galen or Nightingale? *Primary Intention* 1994; 2(1): 14-19.
6. Ayton M. Wound care: wounds that won't heal. *Nurs Times* 1985; 81(46): suppl 16-19.
7. Falanga V, Grinnell F, Gilchrest B, Maddox YT, Moshell A. Workshop on the pathogenesis of chronic wounds. *J Invest Dermatol* 1994; 102(1): 125-27.
8. Kingsley A. A proactive approach to wound infection. *Nurs Stand* 2001; 15(30): 50-58.
9. Collier M. Understanding wound inflammation. *Nurs Times* 2003; 99(25): 63-64.

10. Cutting K, Harding K. Criteria for identifying wound infection. *J Wound Care* 1994; 3(4): 198 201.
11. El-Fadhil E. Omer, E.Y. Arabi, Z . S. Abdein, and M.E. Mekki. The role of bacterial pathogens in pre-operative sepsis of acute mural appendicitis. *East Afr. Med. J.*, 1985, *62* (6).
12. El-Fadhil E. Omer and Suliman, S. I. Bacteriology of acute appendicitis in Khartoum. *East Afr. Med. J., 1979, 56,* (1).
13. Foxman B, Manning SD, Tallman P, et al; Uropathogenic *Escherichia coli* are more likely than commensal *E. coli* to be shared between heterosexual sex partners. *Am J Epidemiol*. 2002; 156(12):1133-40.
14. Foxman B; Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med.* 2002, 8; 113 Suppl 1A:5S-13S.
15. EI-Fadhil E Omer, Ahmed I. EI-Haj. Urinary tract infections in schoolchildren. *Medicine Digest,* 1992, *18,* (6), 3-7.
16. Hooton TM; Recurrent urinary tract infection in women. *Int J Antimicrob Agents*. 2001; 17(4):259-68.
17. El-Haj, K.M. Chugh, T.D. Bacteriuria in the Arabian Gulf. *Arab J. Med.* 1982, *1*, 5-10.