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Short communication

MULTIPLE ANTIMICROBIAL RESISTANCE IN BACTERIAL ISOLATES FROM CLINICAL AND ENVIRONMENTAL SOURCES OF JIMMA HOSPITAL, SOUTH WEST ETHIOPIA

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ABSTRACT:. A total of 545 clinical specimens (pus, blood, urine, and stool) and environmental specimens (air sample, saline solution, nasal swabs etc) were cultured for isolation and identification of aerobic bacteria and antimicrobial susceptibility testing. Out of these, 356(65%) specimens yielded one or more bacterial strains. Frequent bacterial isolates were *S. aureus* (17%), coagulase-negative staphylococci (25%), *Proteus species* (10.%), *Klebsiella species* (8%), *E. coli* and *Enterobacter species* (14%). The antimicrobial susceptibility test result shows that all *E. coli, Klebsiella,* and *Enterobacter species* were resistant to ampicillin. Similarly 93% *S. aureus*, 88% coagulase-negative staphylococci were resistant to the same antimicrobial agent. Eighty five percent of *Klebisiella*, and 79% of *E. coli* were resistant to tetracycline. Almost all the isolates were found to be multiply resistant to the commonly used antimicrobials, ampicillin, tetracycline, chloramphenicol, and trimethoprim-sulphamethoxazole. Antimicrobial resistant strains of bacteria are increasing and may contribute to spread of serious infectious diseases. Therefore, to prevent and control infections by emerging antimicrobial resistant bacterial strains, measures such as strengthening clinical microbiology laboratory, emphasis on hygienic practices in hospital, and prudent use of existing antimicrobial agents are recommended.

Key words/phrases: Antimicrobial resistance, antimicrobial susceptibility, bacterial isolates, microorganisms

INTRODUCTION

Antimicrobial resistant microorganisms have been known from the early days of chemotherapy. The discovery and use of sulphonamide in the 1930s heralded the age of antimicrobial therapy. This drug revolutionized the treatment of many infections; notably those caused by Gram-positive and Gram-negative cocci and were particularly beneficial during the World War II (Finland, 1995). However, sulphonamide resistant organisms such as *Streptococcus pyogens* soon emerged (Levy, 1982). With the advent of penicillin, the search for other naturally occurring antimicrobials was on, and new antibiotics made by soil organisms were isolated and tested.

Thus, came streptomycin, chloramphenicol and tetracycline. Aminoglycosides, such as kanamycin, appeared in the 1950s. Newer ones, such as gentamycine, torbamycine and amikacin were developed in the subsequent twenty years. Bacterial resistance to antibiotics emerged as sulphonamides (Gedde, 1982; McGowan, 1983; Blower, 1993). At first organisms were with low resistance. Through time highly resistant strains, which could not be therapeutically controlled emerged (Levy, 1982; Finland, 1995).

Bacterial resistance to antmicrobials is a threat to public health throughout the world. Patterns of antibiotic use and resistance vary from country to country and even between neighboring hospitals (Gedde, 1982; Blower, 1993). In ideal circumstances antimicrobial treatment is preceded by laboratory identification of infecting microbe and determination of its sensitivity to a range of antimicrobial drugs. But perhaps more often the clinicians must start treatment on a "best guess" basis, because the need for treatment is urgent or laboratory services are not readily available. A “best guess” must then be based on knowledge of the prevalent microbes and the pattern of their antimicrobial susceptibility in the locality (McGowan, 1983; Blower, 1993).

The WHO Scientific working group (WHO, 1978) believed in the need for continued surveillance of antimicrobial susceptibility of all clinical isolates in a particular geographical or institutional setting. Such information can be used locally as a guide for effective treatment, nationally to assist government in formulating a policy for procurement and distribution of antibiotics and internationally to encourage antibiotic manufacturers to be responsible in the marketing and promotion of their products.

In Ethiopia, antimicrobial resistance pattern of bacterial isolates in some hospital settings like those at Black Lion Hospital were recorded (Mesele Gedebou, 1982). Bacteriological survey and antimicrobial susceptibility test of other localities are scarce. A retrospective study of bacterial isolates and antimicrobial sensitivity in Jimma Hospital, reported some seven years ago indicated the prevalence of multi-drug resistant bacterial strains from in-patients and out-patients of the hospital (Zeleke Wolde Tenssay, 1991). Other than that study no published data on the bacterial strains of the locality is available. The objective of the present study is to investigate the frequent bacterial isolates from different clinical and environmental specimens, and determine antimicrobial susceptibility pattern of these isolates.

MATERIALS AND METHODS

Study site: Jimma Hospital from which the different specimens for the study were collected, is a teaching and referral hospital in Jimma, southwest Ethiopia.

Collection of clinical specimens: Clinical specimens (pus, blood, urine, and stool) were collected from in-patients of Medical, Surgical, Paediatrics, Maternity, Obstetrics and Gynaecology wards. The specimens were taken by the attending clinicians or nurses.

Environmental specimens: Samples of surgical instruments like forceps, scissors, were washed with or soaked in sterile saline solution and the same solution was bacteriologically processed for testing sterility of the instruments. Intravenous fluids, and disinfectant solutions, were sampled with sterile syringes and needles. Air samples were taken by exposing Blood Agar in operation rooms for 10 minutes. Nasal swabs were taken from healthy nurses and medical practitioners with sterile cotton swabs soaked in sterile saline.

Bacteriological processing in the laboratory

A total 545 clinical and environmental specimens were collected and bacteriologically analyzed from May 1997 to August 1998. The specimens were cultured within 30 minutes of collection following standard bacteriological procedures recommended by Cheesbrough (1984). Specimens like washings of instruments were inoculated on solid media, and into Tryptone Soya Yeast broth (Difco) for enrichment. After 18-24 hours of incubation at 37° C sub-culture was made from the broth onto solid media. The solid culture media included blood agar, MacConkey agar, nutrient agar, and mannitol salt agar.

Isolation and identification

Bacterial cultures on solid agar media were examined after 18–24 hours of incubation at 37° C. Gram-negative organisms were identified by biochemical and serological tests, and Gram-positive organisms were identified by combinations of colonial morphology, Gram's reactions, catalase, and coagulase tests (Cheesbrough, 1984).

Antimicrobial susceptibility test

Antimicrobial susceptibility tests were done by standard disc diffusion technique described by Bauer *et al.* (1966). Seven and 11 antimicrobial discs (BioMereux) were used for testing Gram-negative bacilli, and Gram- positive cocci, respectively. Standard strains of *E. coli* ATCC 5922 and *Staphylococcus aureus* ATCC 25923 obtained from the National Health and Nutrition Research Institute, Addis Ababa, Ethiopia, were used for controlling inhibition zone.

RESULTS AND DISCUSSION

The survey attempted to examine frequent bacterial pathogens isolated from in-patients of Jimma Hospital. Out of 545 specimens, 356 (65%) yielded one or more pathogens shown in Table 1. Antimicrobial resistance pattern of the isolates was also determined (Table 2). Although the sample size in the present study is relatively small, the information obtained from the study cannot be overlooked since it indicated the extent of antimicrobial resistant bacterial infection in Jimma Hospital. Gram-positive cocci were found to be the most frequent isolates (42%). *S. aureus* was isolated from 34 (56%) of the pus, and 15 (25%) of nasal swabs. *S. aureus* has long been recognized as an important pathogen in human diseases. Carriage of *S. aureus* appears to play an important role in the epidemiology and pathogenesis of infection (Van Belkum *et al.*, 1997). The isolation of *S. aureus* from both clinical and environmental specimens in the present study indicated some of the important sources of staphylococcal infections. This finding is consistent with previous reports from elsewhere (Coast *et al.*, 1998). Coagulase-negative staphylococci in this study were isolated from 89 (25%) of the different clinical and environmental specimens (Table 1). Although CNS is non-pathogenic under normal conditions, the present finding of multi-drug resistant strains from both clinical and environmental specimens in the Jimma Hospital setting may indicate a potential danger of opportunistic infection.

Table 1. Specimens versus bacterial isolates from in-patients of Jimma Hospital, May 1997-August 1998.

Bacterial species	pus n=156	urine n=91	blood n=110	stool n=55	nasal swab n=73	***Environ n=60	No. (%) of all isolates
S. aureus	34(9.5)		8(2.3)	-	15(4.2)	4(1.1)	61 (17)
CNS	26(7.3)	5(1.4)	3(0.5)	-	43((12)	12(3.3)	89 (24.8)
E. coli	14(3.9)	8(2.2)	1(0.2)	1(0.2)*	-	-	24 (6.8)
Klebsiella sp.	11(3.1)	9(2.5)	6(1.7)	-	-	1(0.3)	27 (7.5)
Proteus sp.	25(7)	9(2.5)	-	-	-	-	34 (9.6)
Enterobacter sp.	8(2.2)	14(3.9)	2(0.7)	-	-	-	24 (6.8)
Salmonella sp.	-	-	3(0.8)	2(0.6)	-	-	5 (1.4)
Shigella sp.	-	-	-	1(0.3)	-	-	1 (0.3)
Providencia Sp.	2(0.6)	-	-	-	-	-	2 (0.6)
Pseudomonas sp.	5(1.4)	5(1.4)	1(0.3)	-	-	3(0.8)	14 (3.9)
ß-hemolytic streptococi	2(0.6)	-	-	-	1(0.3)	1(0.3)	4 (1.1)
**Others	5(1.4)	4(1.1)	5(1.4)	51(14.3	4(1.1)	2(0.60)	71 (19.9)
Total	132(37)	54(15.2)	29(8.1)	55(15.4	63(17.7)	23(6.5)	356(100)

Note: *, Enteropathogenic E. coli; **, *Citrobacter sp., Acinetobacter sp., Alpa-streptococci, Bacillus species*, and non-enteric pathogens in case of stool samples; *** Environmental specimens such as air samples, saline solution, samples of surgical instruments, disinfectant solution etc.; and n, number of specimens.

Table 2. Antimicrobial resistance pattern of the most frequent bacterial isolates from in-patients of Jimma Hospital, May 1997-August 1998.

Bacterial isolates	No tested	Am	C	Car	Te	Sxt	Gn	P	Da	K	E	Met
S. aureus	61	56(93)	29(48)	30(50)	42(70)	16(27)	13(22)	54(90)	15(25)	16(27)	23(38)	23(38)
Staph sp.(CNS)	89	78(88)	59(66)	42(47)	67(75)	44(49)	25(28)	72(81)	29(33)	26(29)	36(40)	44(49)
E. coli	24	24(100)	16(67)	21(88)	19(79)	16(67)	7(29)	-	-	9(38)	-	-
Klebsiella sp.	27	27(100)	24(89)	24(89)	23(85)	22(82)	16(59)	-	-	16(59)	-	-
Proteus sp.	34	29(85)	21(62)	24(71)	29(85)	18(53)	13(38)	-	-	15(44)	-	-
Enterobacter sp.	24	24(100)	19(79)	23(96)	22(92)	20(83)	11(46)	-	-	10(42)	-	-
Pseudomonas sp.	14	13(93)	11(79)	13(93)	12(85)	14(100)	7(50)	-	-	10(71)	-	-

Am, ampicillin; P, penicillin; S, streptomycin; Car, carbenicillin; Te, tetracycline; C, chloram phenicol; Sxt, trimethoprim-sulpa methoxazole; Gn, gentamicin; K, kanamycin; E, erythromycin; Met, methicillin; E, erythromycin; Da, clindamycin.

A review of surgical infections (Neu, 1994) recorded that CNS are increasingly important causes of infections particularly in surgical wards. The most frequent Gram-negative bacilli in the present study were, *Proteus sp*. 34(10%), followed by *Klebsiella sp* 27 (8%), *E. coli* 24 (7%), and *Enterobacter sp* 24 (7%). *Proteus sp*. were the most frequently isolated Gram-negative bacilli from pus and discharges. Similarly, *Proteus sp*., Klebsiella sp. and *Enterobacter sp*. were more frequently isolated from urine cultures in the present study. On the other hand, *Klebsiella species* were the most commonly isolated Gram-negative bacilli from blood cultures (Table 1). These *Enterobacteriaceae* groups were the most frequently isolated from clinical diseases in Ethiopia, and other countries (Mesele Gedebou *et al.*, 1988; Neu, 1994; Pitout *et al.*, 1997). Most of these bacterial species are members of the normal intestinal flora and have been commonly isolated from fecal specimens of healthy individuals (Clava *et al.*, 1996). Other studies isolated these bacilli from infant feeds and feeding utensils (Zeleke Wolde Tenssay and Aschalew Mengistu, 1997), and traditional sauces (Mogessie Ashenafi, 1996) from Ethiopia. A retrospective study of urinary pathogens isolated from Tikur Anbasa Hospital (Dawit Wolday and Wondwosen Erge, 1997) reveals that these Gram-negative bacteria comprised approximately 95% of all isolates. The same work reports that *E. coli* and *Klebsiella* sp. were the most common organisms. Although the sample size is small, the rate of isolation of *E. coli* and *Klebsiella sp*. from the urinary tract infections in the present study is comparable to that from Tikur Anbasa Hospital. Other studies isolated these Gram-negative bacilli from nosocomial infections (Mesele Gedebou *et al*., 1987).

A study from Tikur Anbasa Hospital (Eyasu Habte-Gabr *et al*., 1998) indicates that these bacilli to be causes of 90% of hospital acquired infection. The frequency of isolation of these *Enterobactriacea*e from pus and discharges in the present study is comparable to that finding from Tikur Anbasa Hospital.

Most of the bacterial isolates in the present study were multi-drug resistant strains. Antimicrobial susceptibility test of staphylococi isolates in the present study revealed that almost all the isolates were multiply resistant. The percentages of sensitive strains to the commonly prescribed antimicrobial agents (that are penicillin, ampicillin, tetracycline and chloramphenicol) were very low. Forty-three percent of *S. aureus* isolates in this study were methicillin resistant (Table 2). Methicillin resistant *S. aureus* is now a major nosocomial pathogen globally. Moreover, an alarming feature of methicillin resistant *S. aureus* which causes concern clinically is their resistance to so many antimicrobials (Hyvarian *et al*., 1995; Christina and Vanden, 1998). Multi-drug resistant staphylococci have been reported from both developing and developed countries (Hyvarian *et al.*, 1995; Turdge *et al.*, 1996; Christina and Vanden, 1998). Mesele Gedebou (1982) from Tikur Anbasa Hospital, Ethiopia, reported that the percentage of sensitivity of *S. aureus* to penicillin, chloramphenicol, and tetracycline were very low. Similar levels of resistance to penicillin and tetracycline were reported from other developing countries (Chouldhury, 1991; Hyvarian *et al.,* 1995). The frequently isolated Gram-negative rods in the present study were also resistant to commonly used antibacterial agents. All of the *Klebsiella, E. coli* and *Enterobacter* isolates in this study were resistant to ampicillin. A large number of resistance of Gram-negative bacilli to ampicillin has been reported from Yirgalem Hospital, Southern Ethiopia (Ashenafi Belihu and Lindtjorn, 1999), Tikur Anbessa Hospital, Addis Ababa, (Dawit Wolday and Wondwosen Erge, 1997), and elsewhere (Chouldhury, 1991; Dhar and Marafi, 1991). When compared to antimicrobial resistance reported in a previous study from Jimma Hospital (Zeleke Wolde Tenssay, 1991), an increase in frequency of resistant strains of bacteria is observed. Although the present study cannot confirm whether these organisms were hospital strains or not, the multi-drug resistance of the isolates suggest that they could be hospital acquired. Despite the fact that the frequent bacterial isolates in the present observation is multiply resistant to ampicillin, tetracycline, and trimethoprim-sulfamethoxazole, the same anti-microbials are commonly used in Jimma Hospital and in other parts of the country (Zerusenay Desta and Tefera Abula, 1998). A similar practice has been observed in other developing countries (Pitout *et al.,* 1997). This is because alternative antimicrobial agents are either unavailable or too expensive in developing countries, and that drug abuse may be highly practised in this part of the world.

Three factors may be responsible for the development and spread of resistance: mutation in common genes that extends the spectrum of resistance, transfer of resistance genes among diverse microorganisms, and increase in selective pressure in and outside the hospital environment that enhance the development of resistant organisms.

The prevalence of resistant strains of bacteria to commonly available antimicrobials combined with unavailability of alternative antimicrobial in developing countries are contributing to the spread of major infectious diseases, and has been causes of serious epidemics in recent years (Coast *et al.*, 1998). Therefore, appropriate measures need to be taken at an appropriate time to prevent and control infections by emerging antimicrobial resistant bacterial strains. The following measures are recommended if we are to prevent and control such infections: 1) strengthen clinical microbiology laboratory to optimise detection of emerging resistant bacterial strains; 2) enhance continued surveillance of antimicrobial resistance; 3) emphasize hygienic practice in hospital, and 4) creat awareness about prudent use of existing antimicrobial agents.

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