Original Article

BACTERIAL PATHOGENS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY IN GAZA STRIP, PALESTINE

*El-Astal Z**

ABSTRACT:
Objective: To assess common clinically significant isolates and determine their antimicrobial susceptibility patterns.
Design: A retrospective study for bacterial isolates from clinical sources including urine, pus, blood and cerebrospinal fluid. Bacterial susceptibility testing was done by the standardized disk agar diffusion technique.
Setting: The study has utilized microbiology laboratory records in the governmental hospitals of Gaza Strip, Palestine (11 hospitals with 1376 beds) during four different months (January, April, July and October, 2003).
Subjects: A total of 2844 isolates (924 Gram positive and 1920 Gram negative) were scrutinized.
Results: The resistance of *Staphylococcus aureus* was 73.2% to amoxycillin and 1.8% to vancomycin. For *Streptococcus pneumoniae,* 40.4% was resistance to penicillin and 7.4% to erythromycin.
Conclusions: The increasing resistance of organisms indicates that periodic monitoring and possibly modification of empirical therapy are required.

KEY WORDS: Antimicrobial, susceptibility, clinical isolates

Pak J Med Sci October-December 2004 Vol. 20 No. 4 365-370

INTRODUCTION

Worldwide, emergence of antibiotic resistance in all kinds of pathogenic bacteria is a serious public health issue[1-3]. It is associated with greater hospital mortality and longer duration of hospital stay[4], thereby increasing health care costs[5]. Also, colonization and infection with antibiotic-resistant bacteria will make the therapeutic options for infection treatment, extremely difficult or virtually impossible in some instances[6,7].

There are many reasons for this alarming phenomenon, including increasing antibiotic use and misuse in humans, animals and agriculture, clustering and overcrowding, increased elderly population and poor infection control strategies[2,8].

The most common organisms which are leading cause of infections are *E. coli,* coagulase-negative staphylococci, *Klebsiella* spp., *Staph. aureus, Pseud. aeruginosa, Acinetobacter* spp. and *Enterococcus* spp[9,10].

Increasing resistance to penicillin and cephalosporins has become an important issue for one of the most prevalent causes of Gram-positive infection, *Streptococcus pneumoniae*[11,12]. On the other hands, the emergence of vancomycin-resistant *Staph. aureus* and *Enterococcus* spp. has became a major challenge for clinicians

* Dr. Zakaria El-Astal PhD (Microbiology)
Khan Younis Hospital Laboratory,
Khan Younis,
Gaza Strip-Palestinian Authority

Correspondence:

Dr. Zakaria El-Astal
E-mail: zastal@hotmail.com

* Received for publication: April 6, 2004

Accepted: June 1, 2004

treating in many areas of the world[6,13]. However, among Gram-negative bacteria, *E. coli*, has become highly resistant to ampicillin and cotrimoxazole in many countries such as the US and Canada[14], China[9] and Egypt[3]. Also, high resistance of *Pseud. aeruginosa* was recorded in Korea to ceftazidime and gentamicin[15].

Knowledge of local antimicrobial resistance patterns from accurate bacteriological records of culture results may provide guidance towards an empirical therapy before sensitivity patterns are available. The current study was conducted to determine the common clinically significant isolates in Gaza Strip and the antimicrobial susceptibility patterns of these isolates to help policy makers formulate strategies for the rational and effective use of antimicrobial agents.

METHODOLOGY

This is a cross-sectional study for bacterial isolates from clinical sources including urine, pus (wounds exudate and ear discharge), blood and cerebrospinal fluid (CSF) and their antibacterial susceptibility to the antibiotics tested during the year 2003. The study has utilized microbiology laboratory records and analyzed the susceptibilities of all the 16 common clinical bacterial isolates (2844) collected in the governmental hospitals of Gaza Strip (11 hospitals with 1376 beds) during four different months (January, April, July and October, 2003). All specimens were cultured on appropriate media. Significant growth was identified biochemically and serologically in a systematic way according to standard methods[16]. Gram negative bacilli and entercocci were identified to species level by using API 20E and API 32 strips, respectively. Staphylococci were identified by catalase, coagulase, novabiocin, D'Nase and Staph latex tests. Presumptive identification of pneumococci was based on colony morphology, alpha-hemolysis and susceptibility to optochin. Bacterial susceptibility testing was done by the disk diffusion method according to Kirby-Bauer method[17] following the NCCLS assessment criteria[18].

Bacterial inocula were prepared by suspending the freshly grown bacteria in 4-5 ml sterile 0.85% saline and the turbidity was adjusted to that of a 0.5 McFarland standard. The inoculum suspension was spread in three directions on a Mueller Hinton agar plate surface with a sterile swab (except for *Streptococcus* sp., blood agar plates were used). Filter paper disks containing designated amounts of the antimicrobial drugs obtained from commercial supply firms (Sanofi Diagnotic Pasteur) were used.

The antimicrobial disks tested for all isolates were: amoxycillin, 25µg; cephalexin, 30µg; cefuroxime, 30µg; ceftriaxone, 10µg; doxycycline, 30 UI; ciprofloxacin, 5µg and cotrimoxazole, 1.25-23.75µg. Also cloxacillin, 1µg; penicillin, 10 U; erythromycin, 15µg and vancomycin, 10µg were tested against Gram positive bacteria. On the other hands, gentamicin, 30µg and amikacin, 30µg were tested against Gram negative bacteria and nalidixic acid, 30µg was used only against Gram negative uropathogens. The plates were incubated aerobically at 37°C for 18-24 hours.

The patient populations and bacteriological methods used did not change during the study period and the samples did not include multiple isolates from the same patient. The age of the patients and other demographic information were recorded inconsistently and this information was thus not included in the data analysis.

All laboratories tested each organism using the same reagent and antibiotic disks. Controlled strains (*Staph. aureus* ATCC 29213, *Enterococcus faecalis* 29212, *E. coli* ATCC 25922 and *Pseud. aeruginosa* 27853) were included routinely every week for quality control. For data analysis, antibiotic resistance included combined, intermediate and resistant results. Statistical analysis was carried out by using a statistical software package (SPSS).

RESULTS

The 7722 samples collected during the four study months yielded 2844 (36.8%) positive cultures of bacterial growth. Among the different

pathogenic isolates, 469 (16.5%) were detained from outpatients and 2375 (83.5%) from hospitalized patients. The positive samples (n=2844) were cultured from urine (49.2%), pus (39.2%), blood (8.4%) and CSF (3.2%) specimens. Gram-positive cocci contributed 924 (32.5%) isolates and Gram-negative bacilli accounted for 1920 (67.5%) isolates. The most frequently identified pathogens were *E. coli* (32.3%) followed by *Staph. aureus* (19.8%), *Pseud. aeruginosa* (9.3%), *Klebsiella pneumoniae* (8.6%) and *Proteus mirabilis* (8.5%) {Table-I}.

The isolated Gram-negative bacteria showed wide differences in their susceptibility to the tested antimicrobial drugs {Table-II}. The resistance to amoxycillin was 80.1% among *E. coli* and 50.9% among *Haemophilus influenzae*. A high resistance rate to cotrimoxazole was reported among *Acinetobacter haemolyticus* (70.6%). However, *E. coli* resistance to amikacin was only 3.0%. The highest resistance to amikacin was observed among *Pseud. aeruginosa* (8.3%).

Among Gram-positive isolates, *Staph. aureus* resistance to vancomycin was 1.8%. Also, it was 73.2%, 13.8% and 11.7%, to amoxycillin, ceftriaxone and erythromycin, respectively. The resistance of *Strep. pneumoniae* to amoxycillin and penicillin was 61.4% and 40.4%, respectively. The lowest resistance was to erythromycin (7.4%){Table-III}.

In vitro activities of 12 different antibiotics against the bacterial isolates is illustrated in Table-IV. The resistance rates were high among both Gram positive and negative isolates. Resistance to ceftriaxone and ciprofloxacin was higher among Gram positive than Gram negative bacteria. On the other hand, resistance to amoxycillin and cephalexin was higher among Gram negative than Gram positive bacteria.

Table-I: Frequency of bacterial pathogens isolated from different specimen

Isolates	*Urine*	*Pus*	*Blood*	*CSF*	*Among all specimens*
	no. (%)	*no. (%)*	*no. (%)*	*no. (%)*	*no. (%)*
Gram-negative					
E. coli	798 (57.0)	81 (7.3)	33 (13.8)	6 (6.6)	918 (32.3)
Klebsiella pneumoniae	168 (12.0)	60 (5.4)	13 (5.4)	0 (0.0)	246 (8.6)
Proteus mirabilis	129 (9.2)	109 (9.8)	5 (2.1)	0 (0.0)	243 (8.5)
Pseudomonas aeruginosa	81 (5.8)	168 (15.1)	6 (2.5)	9 (9.9)	264 (9.3)
Enterobacter cloacae	54 (3.9)	29 (2.6)	4 (1.7)	0 (0.0)	87 (3.1)
Acinetobacter haemolyticus	27 (1.9)	22 (2.0)	2 (0.8)	0 (0.0)	51 (1.8)
Citrobacter freundii	13 (1.0)	5 (0.4)	0 (0.0)	0 (0.0)	18 (0.7)
Neisseria meningitis	0 (0.0)	0 (0.0)	3 (1.2)	18 (19.8)	21 (0.8)
Serratia marcescens	3 (0.2)	7 (0.6)	5 (2.1)	0 (0.0)	15 (0.5)
Haemophilus influenza	0 (0.0)	14 (1.2)	10 (4.2)	32 (35.2)	51 (1.8)
Morganella morgani	6 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	6 (0.2)
Gram-positive					
Staph. aureus	5 (0.4)	534 (47.9)	25 (10.5)	0 (0.0)	564 (19.8)
Staph. epidermidis	0 (0.0)	0 (0.0)	114 (47.7)	0 (0.0)	114 (4.0)
Staph. saprophyticus	63 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	63 (2.2)
Enterococcus faecalis	52 (3.7)	69 (6.2)	5 (2.1)	0 (0.0)	126 (4.4)
Streptococcus pneumoniae	0 (0.0)	17 (1.5)	14 (5.9)	26 (28.5)	57 (2.0)
Total	1399 (100.0)	1115 (100.0)	239 (100.0)	91 (100.0)	2844 (100.0)

Table-II: Resistance rates (%) of Gram-negative bacilli to antibiotics

Isolates	*Antimicrobial agent*									
	AMX%	*CP%*	*CU%*	*CRO%*	*G%*	*AK%*	*DO%*	*NA%*	*CIP%*	*CO%*
E. coli	80.1	20.8	15.2	11.8	13.4	3.0	59.7	17.6	9.1	58.5
Klebsiella pneumoniae	93.1	27.2	16.7	13.4	21.1	6.5	62.6	21.1	12.6	63.0
Proteus mirabilis	81.5	25.9	17.3	15.2	19.7	5.8	57.2	23.5	16.0	63.0
Pseud. aeruginosa	95.4	89.1	84.6	14.8	26.1	8.3	64.8	58.8	17.8	69.7
Enterobacter cloacae	83.9	31.0	24.1	16.1	23.0	6.9	58.6	20.7	14.9	64.4
Acinet. haemolyticus	96.1	39.2	29.4	17.6	31.4	7.8	66.7	31.4	17.6	70.6
Citrobacter freundii	94.4	27.8	16.7	11.1	16.7	5.5	44.4	22.2	16.7	61.1
Neisseria meningitis	76.2	28.6	19.0	9.5	19.0	4.8	66.7	NT	NT	NT
Serratia marcescens	73.3	26.7	20.0	13.3	13.3	0.0	66.7	20.0	6.7	60.0
Haemophilus influenza	50.9	29.4	17.6	13.7	19.6	3.9	58.8	NT	NT	NT
Morganella morgani	83.3	33.3	16.7	16.7	16.7	0.0	66.7	33.3	16.7	66.7

NT, not tested.
AMX, Amoxycillin; CP, Cephalexin; CU, Cefuroxime; CRO, Ceftriaxone; G, Gentamicin; AK, Amikacin; DO, Doxycycline; NA, Nalidixic acid; CIP, Ciprofloxacin and CO, Cotrimoxazole.

Table-III: Resistance rates (%) of Gram-positive cocci to antibiotics

Isolates	*Antimicrobial agent*									
	P%	*AMX%*	*CP%*	*CU%*	*CRO%*	*DO%*	*CIP%*	*E%*	*CO%*	*VA%*
Staph. aureus	38.9	73.2	19.1	17.5	13.8	65.8	16.1	11.7	68.4	1.8
Staph. epidermidis	51.7	83.3	28.1	21.0	17.5	69.3	19.3	17.5	73.7	0.0
Staph. saprophyticus	48.5	71.4	20.6	13.1	11.1	68.3	14.5	12.0	65.4	0.0
Enter. faecalis	42.1	72.2	24.7	22.6	17.5	72.1	18.2	10.3	69.6	3.2
Strep. pneumoniae	40.4	61.4	26.4	17.3	14.0	58.9	11.3	7.4	60.3	0.0

NT, not tested.
P, Penicillin; AMX, Amoxycillin; CP, Cephalexin; CU, Cefuroxime; CRO, Ceftriaxone; DO, Doxycycline; CIP, Ciprofloxacin; E, Erythromycin; CO, Cotrimoxazole and VA, Vancomycin.

Table-IV: Resistance of antimicrobial agents tested

Drug	*Gram-negative*		*Gram-positive*		*Combined isolates*	
	*No.**	*%*	*No.*	*%*	*No.*	*%*
P	NT	NT	384	41.5	384	41.5
AMX	1611	83.9	679	73.5	2290	80.5
CP	501	26.1	199	21.5	700	24.6
CU	330	17.2	170	18.4	500	17.6
CRO	254	13.2	135	14.6	389	13.7
G	348	18.1	NT	NT	348	18.1
AK	94	4.9	NT	NT	94	4.9
DO	1163	60.6	617	66.8	1780	62.6
NA	390	21.1	NT	NT	390	21.1
CIP	228	12.3	151	16.3	379	13.7
E	NT	NT	111	12.0	111	12.0
CO	1145	62.0	633	68.5	1778	64.1
VA	NT	NT	13	1.5	13	1.5

* No.=Number of resistant isolates NT, not tested.

DISSCUSSION

In this study, the positive bacterial cultures in descending order were isolated from urine, pus, blood and CSF specimens {Table-I}. Similar rates were reported in some countries such as China[9] and Ghana[19].

The most frequently identified pathogens causing infections was *E. coli* followed by *Staph. aureus* and *Pseud. aeruginosa*. This is in the range that is reported in other countries such as China[9] Egypt[3] and Israel[10].

It must be pointed that the comparative results of studies concerning resistance to different antimicrobial agents should take into account the periods when they were conducted and the various clinical parameters of the target population. Moreover, the comparison must consider the limitation of resistance to antimicrobials, which can vary from country to country.

Antimicrobial resistance among Gram-negative bacilli in this study was notable. *E. coli* resistance to amoxycillin was 80.1%, 58.5% to cotrimoxazole, 9.1% to ciprofloxacin and 3.0% to the amikacin. These resistance profiles were less than that reported from Egypt[3]. The high resistance rates of *E. coli* may be due to the mechanism that involve alternations in the outer membrane protein and in the antibiotic efflux system in the cell membrane[20].

Concerning the antimicrobial susceptibility patterns among Gram-positive isolates, the results show that vancomycin resistance of *Staph. aureus* and *Enterococcus faecalis* was 1.8% and 3.2%, respectively {Table-III}. However, many studies worldwide recorded no resistance to this agent among *Staph. aureus* isolates and increased resistance among *Enterococcus faecalis*[3,9]. The reason that a relatively large number of *Staph. aureus* and *Enterococcus faecalis* vancomycin-resistance are being isolated in Gaza Strip needs to be further investigated.

The high rate of resistance to penicillin among *Strep. pneumoniae* (40.4%) in this study is consistent with many authors[3,21,22]. Whereas, lower prevalence was reported from other centers[12,23,24]. Therefore, the rising rates of resistance in *Strep. pneumoniae* afford this pathogen a major impact on the ability to treat some infections.

The results of this study show decreased susceptibility to many antimicrobial drugs used for empiric treatment of infections in Gaza Strip, especially amoxycillin, cotrimoxazole, doxycycline and penicillin. This high rate of resistance is likely due, in part, to the selective pressure resulting from the uncontrolled, unwise and frequent administration of those drugs and by antimicrobial agent policy that permits an easy access of the Palestinian health centers to those agents. This is also associated with the relatively low cost of these antimicrobial agents. Therefore, amoxycillin, cotrimoxazole, doxycycline and penicillin should no longer be prescribed for treatment unless susceptibility tests prove otherwise.

The low resistance to amikacin and erythromycin in this study among Gram- negative and Gram-positive bacteria, respectively suggest that they may still be useful for the treatment of infections by these organisms. However, no antibiotic is a miraculous magic wand against resistant bacteria.

In summary, the resistance to many antimicrobial agents of various isolated pathogenic bacteria is very common in Gaza Strip. Our results implicate that antibiotic resistance in Palestine need to be monitored closely. A national strategy on the limited and prudent use of antibiotics is urgently needed to slow the emergence of antibiotic resistance.

ACKNOWLEDGEMENT

The author wishes to express high appreciation and gratitude to all the laboratory staff who have made every effort in providing the required data needed for this study.

REFERENCE

1. Miller K, O'Neill A & Chopra I. Response of *Escherichia coli* hypermutators to selection pressure with antimicrobial agents from different classes. J Antimicrob Chemother 2002; 49: 925-34.

2. Akhter J, Frayha HH & Qadri SM. Current status and changing trends of antimicrobial resistance in Saudi Arabia. J Med Liban 2000; 48(4):227-32.
3. El Kholy A, Baseem H, Hall GS, Procop GW & Longworth DL.Antimicrobial resistance in Cairo, Egypt 1999-2000: a survey of five hospitals. J Antimicrob Chemother 2003; 51(3):625-30.
4. Goldmann DA, Weinstein RA & Wenzel RP. Strategies to prevent and control the emergence and spread of antimicrobial-resistant microorganisms in hospitals. A challenge to hospital leadership. JAMA 1996;275: 234-40.
5. Snyder JW, McDonald LC & Van Enk R. Common bacteria whose susceptibility to antimicrobials is no longer predictable. J Med Liban 2000; 48(4):208-14.
6. Hand WL. Current challenges in antibiotic resistance. Adolesc Med 2000; 11(2):427-38.
7. Collignon PJ. Antibiotic resistance. Med J Aust 2002; 177(6):325-9.
8. Goossens H. Antibiotic resistance and policy in Belgium. Verh K Acad Geneeskd Belg 2000; 62(5): 439-69.
9. Wang F, Zhu DM, Hu FP & Zhang YY. Surveillance of bacterial resistance among isolates in Shanghai in 1999. J Infect Chemother 2001; 7(2):117-20.
10. Turner D & Dagan R. The sensitivity of common bacteria to antibiotics in children in southern Israel. Harefuah 2001; 140(10):923-9.
11. Chen DK, McGeer A & de Azavedo JC. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. N Engl J Med 1999; 341: 233-9.
12. Jacobs MR, Felmingham D, Appelbaum PC & Gruneberg RN. The Alexander Project Group. The Alexander Project 1998-2000: susceptibility of pathogens isolated from community-acquired respiratory tract infection to commonly used antimicrobial agents. J Antimicrob Chemother. (2003) 52, (2):229-46.
13. Liu HH. Antibiotic resistance in bacteria. A current and future problem. Adv Exp Med Biol 1999; 455: 387-96.
14. Pfaller MA, Jones RN, Doern GV, Kugler K & the Sentry Participant Group. Bacterial pathogens isolated from patients with bloodstream infection: frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY Antimicrobial Surveillance Program (United States and Canada) 1997. J Antimicrob Chemother 1998; 42:1762–70.
15. Lee K, Lee HS, Jang SJ, Park AJ, Lee MH, Song WK & Chong Y. Members of Korean Nationwide Surveillance of Antimicrobial Resistance Group. Antimicrobial resistance surveillance of bacteria in 1999 in Korea with a special reference to resistance of enterococci to vancomycin and gram-negative bacilli to third generation cephalosporin, imipenem, and fluoroquinolone. J Korean Med Sci 2001; 16(3):262-70.
16. Vandepitte, J. El-Nageh, M.M. Tikhomirov, E. Stelling, J.M. and Estrela, A. Guidelines for antimicrobial resistance surveillance. In: WHO Regional Publications, Alexandria-Egypt. Eastern Mediterranean Series (1996) 15, 13-22.
17. Bauer AM, Kirby WMM, Sherris JC & Turck M. Antibiotic susceptibility testing by a standard simple disk method. Am J Clin Pathol 1966; 45:493-6.
18. National Committee for Clinical Laboratory Standards, Performance standards for antimicrobial susceptibility testing. Eleventh Informational Supplement. Document M100-S11. (2001) 21, No. 1. NCCLS, Wayne, Pennsylvania, USA.
19. Ohene A. Bacterial pathogens and their antimicrobial susceptibility in Kumasi, Ghana. East Afr Med J 1997; 74, (7):450-5.
20. Zhang L, Wang F & Zhu DM. Investigation of out membrane barrier mechanism in multiple-antibiotic-resistance *Escherichia coli.* Chin J Infect Dis 1999; 16: 195-7.
21. Tambic T, Tambic A, Kalenic S, Gilic V, Krakar B, Payerl-Pal M et al. Monitoring bacterial resistance to antibiotics in the Croatian Republic. Lijec Vjesn 2000; 122 (7-8):160-4.
22. Hueston WJ & Dickerson L. Antibiotic resistance and the need for the rational use of antibiotics. J Med Liban 2001; 49(5): 246-56.
23. Kanungo R, D'lima D, Rajalakshmi B, Kumar A & Badrinath S. Emerging antibiotic resistant pneumococci in invasive infections in south india: need for monitoring. Indian J Pharmacol 2002; 34: 38-43.
24. Boccia D, Alegiani SS, Pantosti A, Moro ML & Traversa G. The geographic relationship between the use of antimicrobial drugs and the pattern of resistance for Streptococcus pneumoniae in Italy. Eur J Clin Pharmacol 2004; 60(2):115-9.