

Antimicrobial activity against gram negative bacilli from Yaounde Central Hospital, Cameroon

Joseph Gangoue-Pieboji^{1,2*}, Sinata Koulla-Shiro³, Pierre Ngassam², Dieudonne Adiogo³, Peter Ndumbe³

1. - Institute of Medical Research and Medicinal Plants studies, P.O. Box 8404Yaounde, Cameroon

2. - Laboratory of General biology, Faculty of Science, University of Yaounde I, P.O. Box 812Yaounde, Cameroon

3. - Faculty of Medicine and Biomedical Sciences, University of Yaounde I, P.O. Box 1364Yaounde, Cameroon

Abstract

Background: Antimicrobial resistance among bacteria pathogens is a world-wide issue. The antimicrobial susceptibility patterns of common pathogenic bacteria are essential to guide empirical and pathogen-specific therapy; unfortunately, these data are scarce in Cameroon.

Objective: To determine the antimicrobial susceptibility patterns of Gram-negative bacilli isolated in Yaounde Central Hospital Laboratory of Bacteriology.

Methods: Gram-negative bacilli isolates (n = 505), obtained from a wide range of clinical specimens (urine, pus and blood) in Yaounde Central Hospital Laboratory of Bacteriology between March 1995 and April 1998, were evaluated for resistance to antibiotics (amoxicillin, amoxicillin/clavulanate, piperacillin, ceftazidime, cefotaxime, ceftazidime, aztreonam, imipenem, gentamicin, tobramycin, ofloxacin and trimethoprim/sulfamethoxazole) by the Kirby-Bauer disk diffusion method.

Results: High rates of resistance were found in most of the bacteria studied. Resistance to all isolates was mostly observed for amoxicillin (87%), piperacillin (74%) and trimethoprim/sulfamethoxazole (73%). Susceptibilities to third generation cephalosporins (cefotaxime, ceftazidime) and monobactame (aztreonam) were = 91% for *Escherichia coli*, = 71% for *Klebsiella* spp., = 98% for *Proteus mirabilis*, = 50% for *Enterobacter* spp. and *Citrobacter* spp. *Pseudomonas aeruginosa* was less susceptible to cefotaxime (2%) and aztreonam (33%), and highly susceptible to ceftazidime (72%) whereas *Acinetobacter baumannii* was highly resistant to aztreonam (100%), to cefotaxime (96%) and ceftazidime (62%). Imipenem (98%) was the most active antibiotic followed by the ofloxacin (88%). Susceptibility of all isolates to gentamicin was 67%.

Conclusion: These results indicate that surveillance to antimicrobial resistance in Cameroon is necessary to monitor microbial trends, antimicrobial resistance pattern, and provide information for choosing empirical or direct therapy to physicians.

Key words: antimicrobial agents, resistance, Gram-negative bacilli, bacteria susceptibility testing, Cameroon.

African Health Sciences 2006; 6(4):232-235

Introduction

Gram-negative bacilli are the most important bacterial pathogen, and are generally resistant to antibiotics^{1,2,3}. Monitoring for antimicrobial resistance in this group is important because resistance has been reported to be associated with increased patient morbidity and mortality, and contributed to escalating healthcare cost⁴. Antimicrobial resistance is increasing in many bacteria and is a worldwide problem^{5,6}.

The antimicrobial susceptibility patterns of common pathogenic bacteria are essential to guide empirical and pathogen-specific therapy. This informa-

tion is also important for rational policies against antimicrobial resistance. Unfortunately, in many developing countries, these data are scarce because of dwindling resources. In Cameroon, data on antimicrobial resistance among bacterial pathogens are sparse. In an effort to determine the extent of antimicrobial resistance amongst Gram-negative bacilli isolated in Yaounde Central Hospital Laboratory of Bacteriology, we conducted a survey of 505 Gram-negative bacilli and measured their in vitro susceptibility to antimicrobial agents.

Materials and Methods

Between April 1995 and March 1998, all the aerobic Gram-negative bacilli isolated in the laboratory of bacteriology of Yaounde Central Hospital were collected prospectively. The isolates studied were confined to unrelated first isolates from different patients, and did

Corresponding author

Institute of Medical Research and Medicinal plants Studies (IMPM)

P. O. Box 8404Yaounde, Cameroon

Tel: 237 924 57 70

Fax: 237 222 62 62

[E-mail: jgangoue@yahoo.fr](mailto:jgangoue@yahoo.fr)

not include multiple isolates from the same patient. Isolates were recovered from urine, pus and blood cultures and identified by standard laboratory technique methods⁷ and confirmed by Api 20E (BioMerieux, France).

The antimicrobial susceptibility test was determined by the Kirby-Bauer disk diffusion method following the National Committee of Clinical Laboratory Standards (NCCLS) for agar diffusion tests⁸. The antibiotics tested were amoxicillin (30µg), amoxicillin/clavulanate (20/10 µg), piperacillin (100 µg), imipenem (10 µg), cefazolin (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg), gentamicin (10 µg), tobramycin (10 µg), ofloxacin (5 µg) and trimethoprim/sulfamethoxazole (1.25/23.75 µg). The following American Type Culture Collection (ATCC) microorganisms were tested each time susceptibility testing was performed: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Test results were only validated in the cases where inhibition zone diameters of the control strains were within performance ranges⁸.

Data were analyzed using Whonet 4 (World Health Organization, Geneva, Switzerland) and resistance included combined, intermediary and resistance results.

Results

A total of 505 aerobic isolates were collected, identified, and tested. Enterobacteriaceae (*E. coli*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., and *Citrobacter* spp.) represented 79.8% of the isolated strains. Non

fermentative Gram negative bacilli (*Pseudomonas* spp. and *Acinetobacter baumannii*) represented 20.2%. The sources of the isolates are shown in table 1.

Table 2 summarizes the results of susceptibility tests of Gram-negative bacilli studied against antimicrobial agents. Imipenem (98% susceptible (S)) was the most active agent against all pathogens tested followed by ofloxacin (88% S) and ceftazidime (86% S). Amoxicillin (13% S), piperacillin (26% S), trimethoprim/sulfamethoxazole (27% S), cefazolin (30% S) and amoxicillin/clavulanate (37% S) were the least active agents.

Against *E. coli*, susceptibility rates range from 15% (amoxicillin, piperacillin) to 99% (imipenem). The least active agents against *Klebsiella* spp. were amoxicillin (0% S) followed by piperacillin (10% S) and trimethoprim/sulfamethoxazole (15% S). The most active agents were imipenem (93% S) and ofloxacin (98% S). *Proteus mirabilis* was the most sensitive pathogen of all microorganisms. The susceptibility rate ranged from 40% (amoxicillin) to 100% (imipenem, ceftazidime and aztreonam). Concerning *Enterobacter* spp., only two antimicrobial agents had activity rate >80% ((ofloxacin (98%) and imipenem (97%)). For *Citrobacter* spp. the most active agents were imipenem (96% S) followed by ceftazidime (76% S) and ofloxacin (73% S). Only 94% of *P. aeruginosa* isolates were susceptible to imipenem, as compared to 100% observed for *A. baumannii*. However, 72% of *P. aeruginosa* were susceptible to ceftazidime as compared to only 38% of the *A. baumannii*. All the *A. baumannii* isolates tested were resistant to aztreonam.

Table 1. Distribution (%) of bacterial species by clinical specimen

Organism	Clinical specimen		
	Pus	Urine	Blood
<i>Escherichia coli</i>	33.7	65.0	1.3
<i>Klebsiella</i> spp.	43.3	44.3	12.4
<i>Proteus mirabilis</i>	58.0	42.0	0.0
<i>Enterobacter</i> spp.	47.5	42.5	10.0
<i>Citrobacter</i> spp.	50.0	50.0	0.0
Indole positif <i>Proteus</i> spp.	50.0	43.8	6.2
<i>Pseudomonas aeruginosa</i> .	71.6	22.4	6.0
<i>Acinetobacter baumannii</i>	54.2	25.0	20.8
<i>Pseudomonas</i> spp.	70.0	10.0	20.0

Table 2. Susceptibility (%) of Gram negative bacilli studied

	All isolates n=505	<i>E. coli</i> n=163	<i>Klebsiella</i> spp. n=97	<i>P. mirabilis</i> n=62	<i>Enterobacter</i> spp. n=40	<i>Citrobacter</i> spp. n=26	Indole positif <i>Proteus</i> spp. n=16	<i>P. aeruginosa</i> n=67	<i>A. baumannii</i> n=24	<i>Pseudomonas</i> spp. n=10
Isolates										
Antibiotic	%S	%S	%S	%S	%S	%S	%S	%S	%S	%S
Amoxicillin	13	15	0	40	0	0	0	/	/	/
Amoxicillin / clavulanate	37	43	35	69	0	40	0	/	/	/
Piperacillin	26	15	10	53	35	19	56	46	4	50
Cefazolin	30	41	24	48	0	4	0	/	/	/
Cefoxitin	74	91	84	97	5	8	31	/	/	/
Cefotaxime	67	91	71	98	60	50	94	2	4	30
Ceftazidime	86	95	88	100	80	76	94	72	38	70
Aztreonam	73	92	75	100	65	69	88	33	0	20
Imipenem	98	99	99	100	97	96	94	94	100	100
Gentamicin	67	84	65	68	60	58	50	49	58	50
Tobramycin	57	^a	/	/	/	/	/	56	69	50
Ofloxacin	88	91	98	98	98	73	82	66	70	90
Trimethoprim/sulfamethoxazole	22	22	15	55	43	19	6	/	/	/

^a not tested

Discussion

This study represents an extensive examination of the susceptibility patterns of Gram negative bacilli isolated in the laboratory of bacteriology of Yaounde Central Hospital. Our isolates represent both nosocomial and community acquired-pathogens, and were collected from April 1995 to March 1998. The results of this study show that in general, high rate of resistance occurs among Gram-negative bacilli to commonly used antibiotics (penicillins, first generation cephalosporin and trimethoprim/sulfamethoxazole).

For *E. coli* and *Klebsiella* spp., the rate of resistance to third generation cephalosporins and other β -lactam antibiotics can be explained by the high production of penicillinase and the production of extended spectrum β -lactamase^{3,9}. However, the rate of this resistance is high compared to those reported in developed countries^{10,11}.

Enterobacter spp. and *Citrobacter* spp. were highly resistant to ceftazidime, cefotaxime and aztreonam. Similar results were observed in Cairo (Egypt) by El Kholy *et al.*, 2003¹² and in developed countries^{10,13}. This resistance could be explained by the high production of cephalosporinase and the production of extended spectrum β -lactamase by these strains^{3,9}.

Our data showed that *P. mirabilis* is the most sensitive species. The susceptibility rates of this species to all antibiotics tested are compared to those observed in developed countries^{10,11}.

Non- β -lactam antibiotics resistance rates among the species of the *Enterobacteriaceae* family studied were comparable to the reported rates in other parts of developing countries^{14,15}, but higher than those reported in developed countries^{10,13}. The combination of trimethoprim/sulfamethoxazole is extensively used in Africa owing to its antimicrobial spectrum of activity, and its low cost¹⁶. In addition, extended spectrum β -lactamase production is usually associated with resistance to non- β -lactam antibiotics such as aminoglycosides, fluoroquinolones and trimethoprim/sulfamethoxazole¹⁷.

The susceptibility rates of isolates of *P. aeruginosa* and *A. baumannii* in this study to all antibiotics tested except for imipenem, were low compared to those reported in developed countries^{10,18,19} and similar to those observed in Egypt¹² and in West Africa¹⁵.

In conclusion, our study suggests the high rates of antimicrobial resistance among Gram-negative bacilli. The presence of *E. coli* and *Klebsiella* spp. isolates resistant to third generation cephalosporin suggests the importance of monitoring this phenotype. Particularly alarming is the appearance of low level imipenem resistance among different species of Gram negative bacilli studied. The results of this study indicate that more resources should be allocated to encourage good antibiotics utilization and practice. In addition, to provide information for choosing either empirical or direct therapy to physicians, surveillance to antimicrobial resistance is necessary.

Acknowledgements

We thank the Yaounde Central Hospital administration for accepting us to conduct this study in their hospital. We also thank the technicians of the Laboratory of Bacteriology of the Yaounde Central Hospital Mrs J. Mvondo, M. Tchouko, S. Ntoul, E. Dongmou, M. Wodo, M. Abiazan and M.C. Epape for their help during this work and Drs G. Kuepouo and A. Tchinda for reading the manuscript.

References

1. Diekema DJ, Pfaller MA, Jones RN, Doern GV, Winokur PL, Gales AC et al. Survey of bloodstream infections due to gram-negative bacilli: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, and Latin America for the SENTRY antimicrobial surveillance program, 1997. *Clin Infect Dis* 1999; 29: 595-607.
2. Livermore DM. β -lactamases in laboratory and clinical resistance. *Clin Microb Rev* 1995; 8: 557-584.
3. Gangoué-Piéboji J. Résistance des bacilles à gram-négatif aux antibiotiques: prévalence et caractérisation des β -lactamases à spectre élargi à l'hôpital Central de Yaoundé. Thèse de Doctorat de 3^e cycle, Université de Yaoundé I, 2000 : P 210.
4. Cosgrove SE, Kaye KS, Eliopoulos GM and Carmeli Y. Health and economic outcomes of the emergence of third-generation cephalosporin resistance in *Enterobacter* species. *Arch Intern Med* 2002; 162: 185-190.
5. Wise R, Hart T, Cars O, Streuleus M, Helmut, Huovinen P. Antimicrobial resistance is a major threat to public health. *British Med J* 1998; 317: 609-610.
6. Shas DM, Gerdin DN, John JF, Jr, Craig WA, Borstein DL, Duncan RA et al. Society for Healthcare Epidemiology of America and Infectious Diseases of America Committee on the prevention of antimicrobial resistance. *Clin Infect Dis* 1997; 25: 584-599.
7. Farmer II JJ, Kelly MT. Enterobacteriaceae. In Lenette EH, Balows A, Hausler WJ, Shadomy HJ (eds). *Manual of clinical Microbiology*. Washington DC: American Society for Microbiology; 1991, P. 360-383.
8. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2 A6 (M100-S7). Wagne, PA: NCCLS, 1997.
9. Gangoué-Piéboji J, Koulla-Shiro S, Ngassam P, Adio D, Njine T, Ndumbe P. Antimicrobial resistance of Gram-negative bacilli isolates from inpatients and outpatients at Yaounde Central Hospital, Cameroon. *Inter J Infect Dis* 2004; 8: 147-154.
10. Wenzel RP, Sahn DF, Thornsberry C, Draghi DC, Jones ME, Karlowsky JA. In vitro susceptibilities of gram-negative bacteria isolated from hospitalized patients in four European countries, Canada, and the United States in 2000-2001 to expanded spectrum cephalosporin and comparator antimicrobials: Implications for therapy. *Antimicrob Agents Chemother* 2003; 47: 3089-3098.
11. Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO-SENS project. *J Antimicrob Chemother* 2003; 51: 69-76.
12. 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: 625-630.
13. Karlowsky JA, Jones ME, Thornsberry C, Friedland IR, Sahn DF. Trends in antimicrobial susceptibilities among enterobacteriaceae isolated from hospitalized patients in the United States from 1998 to 2001. *Antimicrob Agents Chemother* 2003; 47: 1672-1680.
14. Lamikamra A, Ndep RB. Trimethoprim resistance in urinary tract pathogens in two Nigeria hospitals. *J Antimicrob Chemother* 1989; 23: 151-154.
15. Koulla-Shiro S, Boye CS, Dosso M, the members of the palm project. Surveillance of antimicrobial susceptibility of gram-negative pathogens responsible for nosocomial infections in West Africa (Abstract number E-95). San Diego, California: 42nd ICAAC; 1998, P 197.
16. Huovinen P, Sundström L, Swedberg G, Sköd O. Trimethoprim and sulfonamide resistance. *Antimicrob Agents Chemother* 1995; 39: 279-289.
17. Gales AC, Bolmstrom A, Sampaio J, Sader HS. Antimicrobial susceptibility of *Klebsiella pneumoniae* producing extended spectrum β -lactamase (ESBL) isolated in hospital in Brazil. *Brazilian J Infect Dis* 1997; 1: 196-203.
18. Jones RN, Sader HS, Beach ML. Contemporary in vitro spectrum of activity summary for antimicrobial agents tested against 18569 strains non-fermentative gram-negative bacilli isolated in the SENTRY antimicrobial surveillance program (1997-2001). *Inter J Antimicrob Agents* 2003; 22: 551-556.
19. Karlowsky JA, Draghi DC, Jones ME, Thornsberry C, Friedland IR, Sahn DF. Surveillance for antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United States 1998 to 2001. *Antimicrob Agents Chemother* 2003; 47: 1681-1688.