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Antimicrobial resistance of Gram-negative bacilli isolates from inpatients and outpatients at Yaounde Central Hospital, Cameroon

Joseph Gangoué Piéboji[a,*], Sinata Koulla-Shiro[b], Pierre Ngassam[a], Dieudonné Adiogo[b], Thomas Njine[a], Peter Ndumbe[b]

[a] *Laboratory of General Biology, Faculty of Science, University of Yaounde I, P.O. Box 812 Yaounde, Cameroon*
[b] *Faculty of Medicine and Biomedical Sciences, University of Yaounde I, P.O. Box 1364 Yaounde, Cameroon*

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Resistance phenotype

Summary *Objective:* To determine and compare antimicrobial susceptibility patterns of pathogenic bacteria from inpatients and outpatients at a university teaching hospital in Yaounde, Cameroon.

Methods: Gram-negative bacilli isolates ($n = 522$), obtained from a wide range of clinical specimens (urine, pus and blood) from inpatients and outpatients at Yaounde Central Hospital between March 1995 and April 1998, were evaluated for resistance to antibiotics (amoxicillin, amoxicillin/clavulanate, piperacillin, cefazolin, cefoxitin, cefotaxime, ceftazidime, aztreonam, imipenem, gentamicin, tobramicin, ofloxacin and trimethoprim/sulfamethoxazole).

Results: Of the 522 isolates recorded, 80.3% were Enterobacteriaceae. A high incidence of resistance to amoxicillin (85%), piperacillin (75%) and trimethoprim/sulfamethoxazole (71%) was observed. The proportion of antimicrobial-resistant isolates from inpatients was significantly higher than that from outpatients ($P < 0.05$), except for piperacillin, tobramicin and trimethoprim/sulfamethoxazole. The combinations of antimicrobial and organism showed that the percentage of ceftazidime-resistant *Pseudomonas aeruginosa* and ceftazidime-resistant *Enterobacter cloacae* were 26.8% and 24% respectively. The rate of antimicrobial resistance in isolates from inpatients was not significantly higher than that in isolates from outpatients for all the antimicrobial/organism combinations, except for ceftazidime-resistant *Escherichia coli*, which was exclusively found in isolates from inpatients. Among Enterobacteriaceae, high and low level penicillinase (mostly in *E. coli* (13.6% and 11% respectively) and *Klebsiella* spp. (9% and 8% respectively) were the most important β-lactam resistance phenotypes (31.2% and 23.6%, respectively). Wild type (exclusively observed in *E. coli*, *Proteus mirabilis* and *Salmonella* spp.) and low level penicillinase

*Corresponding author. Present address: University of Liege, Centre for Proteins Engineering, Laboratory of Enzymology, Institute of Chemistry B6, Sant-Tilman, B 4000 Liege, Belgium. Tel.: +32-4-366-35-04; fax: +32-4-366-33-64.
E-mail address: jgangoue@yahoo.fr (J.G. Piéboji).

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were higher in outpatient than inpatient isolates (wild type—17.9% vs 10.8% and low level penicillinase—29.4% vs 20.5%, respectively; $P < 0.05$). However, extended spectrum β-lactamase strains (*Klebsiella* spp. (3.5%), *E. coli* (2.6%), *Citrobacter* spp. (0.7%), *Enterobacter* spp. (0.4%) and *P. mirabilis* (0.2%)) were exclusively recovered from inpatients. Penicillinase and high level cephalosporinase resistance phenotypes were frequently observed in non-fermenter Gram-negative bacilli (46.6% and 29.1% respectively). However, there were no significant differences in penicillinase and cephalosporinase resistance between inpatient and outpatient isolates.

Conclusion: As the incidence of antimicrobial resistance is substantially higher in isolates from inpatient than outpatient pathogens, more resources should be allocated within the hospital to encourage good antibiotic practices and good hospital hygiene.
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Introduction

Antimicrobial-resistant bacteria are the cause of numerous clinical problems worldwide. The development and the increase of antimicrobial resistance among microbial pathogens causing nosocomial and community-acquired infections is known to be associated with the level of antibiotic use.[1] Most studies have found a higher prevalence of antimicrobial resistance in hospitals than in the community.[2–4] The strategy for the control of antimicrobial resistance lies mainly in the implementation of effective infection control measures and antibiotic auditing. For these measures, it is important to have data on the identification and resistance patterns of clinical bacteria, and to know the magnitude of antimicrobial resistance in hospitals compared with that in the community. These data may help to establish preventive and therapeutic guidelines for clinicians when appropriate.[5,6]

This study was designated to evaluate and to compare the resistance of Gram-negative bacilli causing nosocomial and community-acquired infections in Yaounde Central Hospital, Cameroon.

Materials and methods

Study design and patients

To monitor the antimicrobial resistance patterns of nosocomial and community-acquired infections, relevant data from Yaounde Central Hospital (YCH) concerning Gram-negative bacilli isolated from inpatients and outpatients were analysed. The study was carried out prospectively between March 1995 and April 1998. YCH is a university teaching hospital with about 500 beds containing several departments, including: medicine, surgery, intensive care, obstetrics/gynaecology, paediatrics and emergency. Patients included in this study were registered on a study investigation form with details of name, age, sex, hospital department, date of admission, diagnosis on admission, antibiotic prophylaxis or treatment and clinical specimens. This form was completed with the results of laboratory diagnosis and data of antimicrobial susceptibility tests. After laboratory diagnosis, we excluded patients without Gram-negative bacilli.

Bacterial isolates

A total of 522 Gram-negative bacilli isolates isolated between 1995 and 1998 were obtained from a wide range of clinical specimens including urine, pus and blood from inpatients and outpatients. Only one isolate per patient was studied. Organisms were identified by conventional methods[7] and confirmed by API 20E (bioMérieux France). Nosocomial infections were diagnosed on the basis of clinical and laboratory data. Isolates recovered from patients at least 48 hours after admission were considered nosocomial. Community-acquired infections were diagnosed on admission and from ambulatory care. The isolates studied included *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca*, *Proteus mirabilis*, indole positive *Proteus* spp., *Providencia* spp., *Salmonella* spp., *Shigella* spp., *Pseudomonas* spp., *Acinetobacter baumannii* and *Flavobacterium meningosepticum*.

Susceptibility testing

Antimicrobial susceptibilities were determined by Kirby—Bauer disk diffusion following the definition of National Committee of Clinical Laboratory Standards (NCCLS) for agar diffusion tests.[8] The antibiotics tested were amoxicillin, amoxicillin/clavulanate, piperacillin, imipenem, cefazolin, cefoxitin, cefotaxime, ceftazidime, gentamicin, tobramycin, ofloxacin, and trimethoprim/sulfamethoxazole.

E. coli American Type Culture Collection (ATCC) 25922 and *P. aeruginosa* ATCC 27853 were tested along with the isolates for quality control purposes. Test results were only accepted when inhibition zone diameters of the control strains were within performance ranges.[8] The data were stratified by all inpatients and all outpatients.

Antimicrobial/organism combinations and β-lactam resistance phenotype

We studied the following antimicrobial/organism combinations, taking into account their current and potential clinical importance based on the results of antimicrobial susceptibility tests. These combinations included: ceftazidime/*E. coli*; ceftazidime/*Klebsiella* spp.; ceftazidime/*Enterobacter cloacae*; ceftazidime/*Pseudomonas aeruginosa*; imipenem/*P. aeruginosa*.

The β-lactam resistance phenotypes were determined using the results of antimicrobial susceptibility tests.[9,10] Resistance phenotypes were defined as follows:

For Enterobacteriaceae:

- Wild type: strain susceptible to all β-lactams used.
- Penicillinase phenotype: strains resistant to amoxicillin and piperacillin and moderately resistant to cefazolin.
- High level penicillinase: strains resistant to amoxicillin, amoxicillin/clavulanate, piperacillin and cefazolin.
- Inhibitor-resistant TEM phenotype: strains resistant to amoxicillin, amoxicillin/clavulanate and piperacillin.
- Low level cephalosporinase: strains resistant to amoxicillin, amoxicillin/clavulanate, cefazolin, and cefoxitin.
- High level cephalosporinase: strains resistant to amoxicillin, amoxicillin/clavulanate, piperacillin, cefazolin, cefoxitin, moderately resistant to cefotaxime, ceftazidime and aztreonam.
- Extended spectrum β-lactamase: strains resistant to amoxicillin, amoxicillin/clavulanate, piperacillin, cefazolin, cefotaxime, ceftazidime and aztreonam. We confirmed this phenotype by the double disk synergy test.[11]

For non-fermenter Gram-negative bacilli:

- Penicillinase: strains resistant or moderately resistant to piperacillin, cefotaxime and aztreonam.
- High level cephalosporinase: strains resistant to piperacillin, cefotaxime, aztreonam and ceftazidime.
- Low level cephalosporinase: strains resistant to cefotaxime.

As with the data of susceptibility tests, the data of β-lactam resistance phenotype were stratified by all inpatients and all outpatients. The β-lactam resistance phenotype of organisms from hospitalised patients was compared with that of isolates from outpatients.

Statistics

Data were analysed using Whonet 4 (World Health Organisation, Geneva, Switzerland) and Epi Info version 6.04c (Centers for Disease Control and Prevention, Atlanta, GA, USA). The Mantel-Haenszel chi-squared test was used and the 2-tailed Fisher's exact test was used when an expected value was less than 5. A P value of less than 0.05 was considered significant.

Results

Enterobacteriaceae constituted 80.3% of the 522 isolates. The distribution of bacterial species by clinical specimens and origin is presented in Table 1. *E. coli* was the predominant pathogen isolated from both inpatients and outpatients, representing 25.9% and 43.4% of all isolates, respectively. The majority of isolates (67.4%) were nosocomial. From inpatients, pathogens were mostly isolated from pus (50.9%) while the outpatient strains were mostly from urine (56.5%).

The results of susceptibility to antibiotics of different strains showed that the highest rates of resistance (resistant plus intermediate) were to amoxicillin (85%), piperacillin (73%) and trimethoprim/sulfamethoxazole (71%) (Table 2).

Except for piperacillin, tobramicin and trimethoprim/sulfamethoxazole, the occurrence of resistance in isolates from inpatients was significantly higher than that in isolates from outpatients; for trimethoprim/sulfamethoxazole, the occurrence of resistance in isolates from outpatients was higher than that in isolates from inpatients (Table 3).

In all, 419 isolates of Enterobacteriaceae were collected (80.3%). The incidence of resistance to amoxicillin, amoxicillin/clavulanate, piperacillin, cefazolin and trimethoprim/sulfamethoxazole were 85%, 62%, 73%, 69% and 71% respectively (Table 2). More than 85% were susceptible to third

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

Bacterial species ($n = 522$) (%)	Inpatient $n = 352$ (67.4)	Outpatient $n = 170$ (32.6)	Clinical specimens					
			Urine		Pus		Blood	
			Inpatient $n = 145$ (41.2)	Outpatient $n = 96$ (56.5)	Inpatient $n = 179$ (50.9)	Outpatient $n = 68$ (40.0)	Inpatient $n = 28$ (8.0)	Outpatient $n = 6$ (3.5)
Escherichia coli	25.9	43.4	37.9	53.1	19.0	30.9	7.1	0.0
Klebsiella spp.	18.2	19.1	18.6	16.7	15.6	20.6	32.1	50.0
Proteus spp.	15.9	12.7	14.5	12.5	19.0	14.7	3.6	0.0
Enterobacter spp.	8.8	5.2	8.3	5.2	8.9	4.4	10.7	16.7
Citrobacter spp.	4.8	5.2	6.2	4.2	4.5	7.4	0.0	0.0
Pseudomonas spp.	17.9	8.7	9.0	3.1	25.7	14.7	14.3	33.3
Acinetobacter baumannii	5.7	2.3	2.8	2.1	6.1	2.9	17.9	0.0
Others[a]	2.8	3.5	2.8	3.0	1.2	4.4	14.3	0.0

n: number of strains.
[a] *Serratia* spp., *Salmonella* spp., *Shigella* spp. and *Flavobacterium meningosepticum*.

Table 2 Antimicrobial susceptibility (%) of 522 strains of Gram-negative bacilli.

Antibiotics	Breakpoints	Number of isolates tested	Susceptibility (%)		
			Resistant	Intermediate	Susceptible
Amoxicillin[a]	14–17	419	83	2	15
Amoxicillin/clavulanate[a]	14–17	419	30	32	38
Piperacillin	18–20	522	65	8	27
Cefazolin[a]	15–17	419	53	16	31
Cefoxitin[a]	15–17	419	20	6	74
Cefotaxime	15–22	522	15	18	67
Ceftazidime	15–17	522	9	5	86
Aztreonam	16–21	522	16	11	73
Imipenem	14–15	522	1	1	98
Gentamicin	13–14	522	30	3	67
Tobramycin[b]	13–14	96	42	2	56
Ofloxacin	13–15	522	9	2	89
Trimethoprim/sulfamethoxazole[a]	11–15	419	69	2	29

[a] Only tested against Enterobacteriaceae.
[b] Only tested against non-fermenter Gram-negative bacilli.

generation cephalosporins, ofloxacin and imipenem. As for Gram-negative bacilli, the inpatients' isolates presented a significantly higher rate of antimicrobial resistance than the outpatients' isolates, except for imipenem and trimethoprim/sulfamethoxazole (1.9% vs 0%, 70.9% vs 72.8%, respectively). For non-fermenter Gram-negative bacilli, a total of 103 isolates were collected (19.7%). High rates of resistance were observed to cefotaxime, aztreonam and piperacillin (95%, 77% and 64%, respectively). A high proportion of isolates were susceptible to imipenem (95%) whereas only 70% were susceptible to ofloxacin and 63% to ceftazidime. However, the proportion of antimicrobial resistance in isolates from inpatients was not significantly higher than that in isolates from outpatients except cefotaxime (96.4% vs 10.5%, respectively, $p < 0.001$).

Many phenotypes of resistance to β-lactams (wild type, low level penicillinase, high level penicillinase, inhibitor-resistant TEM, low level cephalosporinase, high level cephalosporinase and extended spectrum β-lactamase) were identified. For Enterobacteriaceae, high and low

Table 3 Antimicrobial resistance in Gram-negative bacilli isolates from inpatients vs outpatients.

Antibiotics	Number of resistant isolates/total of number of isolates tested (%)		P value
	Inpatients	Outpatients	
Amoxicillin	239/268 (89.2)	120/151 (79.5)	<0.01
Amoxicillin/clavulanate	182/268 (67.9)	79/151 (52.3)	<0.01
Piperacillin	264/352 (75.0)	116/170 (68.2)	0.10[a]
Cefazolin	201/268 (75.0)	88/151 (58.2)	<0.01
Cefoxitin	86/268 (32.1)	26/151 (17.2)	<0.01
Cefotaxime	141/352 (40.0)	32/170 (18.8)	<0.01
Ceftazidime	63/352 (17.9)	14/170 (8.2)	<0.01
Aztreonam	120/352 (34.1)	21/170 (12.3)	<0.01
Imipenem	11/352 (3.1)	0/170 (0.0)	0.01
Gentamicin	137/352 (38.9)	32/170 (18.8)	<0.01
Tobramycin	36/77 (46.7)	5/19 (26.3)	0.10[a]
Ofloxacin	53/352 (15.0)	7/170 (4.1)	<0.01
Trimethoprim/sulfamethoxazole	190/268 (70.9)	110/151 (72.8)	0.67[a]

[a] Not significant.

Table 4 Resistance to specific antimicrobials in isolates from inpatients vs outpatients for antimicrobial/pathogen combinations.

Antimicrobial/pathogen combination	Number of resistant isolates/total of number of isolates tested (%)		P value
	Inpatients	Outpatients	
Ceftazidime/*Escherichia coli*	9/91 (9.9)	0/72 (0.0)	<0.01
Ceftazidime/*Klebsiella pneumoniae*	9/64 (14.1)	2/30 (6.7)	0.92[a]
Ceftazidime/*Enterobacter cloacae*	5/22 (22.7)	1/3 (33.3)	0.58[a]
Ceftazidime/*Pseudomonas aeruginosa*	16/56 (28.6)	2/11 (18.2)	0.73[a]
Imipenem/*Pseudomonas aeruginosa*	4/56 (7.1)	0/11 (0.0)	0.82[a]

[a] Not significant.

level penicillinase, mostly observed in *E. coli* (13.6% and 11%), and *Klebsiella* spp. (9% and 8%) were the most important resistance phenotypes (31.2% and 23.6% respectively). Wild-type (only observed in *E. coli* (6%), *P. mirabilis* (6%) and *Salmonella* spp. (1%)), and low level penicillinase phenotypes were recovered in significantly higher numbers from outpatients' than from inpatients' isolates (wild type—17.9% vs 10.8% and low level penicillinase 29.1% vs 20.5%, respectively $p < 0.05$). However, extended spectrum β-lactamase-producing strains (*Klebsiella* spp. (3.5%), *E. coli* (2.6%), *Citrobacter* spp. (0.7%), *Enterobacter* spp (0.4%) and *P. mirabilis* (0.2%)) were exclusively recovered from inpatients (11.9%, $p < 0.001$).

For non-fermenter Gram-negative bacilli, penicillinase (*Pseudomonas* spp. (37.8%), *Acinetobacter baumannii* (8.7%)) and high level cephalosporinase (*Pseudomonas* spp. (15.5%) and *A. baumannii* (13.6%)) were the most frequent resistance phenotypes (46.6% and 29.1% respectively). Low level cephalosporinase (observed in *Pseudomonas* spp. — 18.4% of isolates) was more frequently observed in outpatients' than inpatients' isolates (26.3% vs 17.8%) as was high level cephalosporinase (31.6% vs 28.6%) while penicillinase was more frequently found in isolates from inpatients than outpatients (48.8% vs 42.1%). However, the difference between these isolates was not significant.

For antimicrobial/organism combinations the proportion of ceftazidime-resistant *P. aeruginosa* and ceftazidime-resistant *E. cloacae* were 26.8% and 24% respectively. The proportion of antimicrobial resistance in isolates from inpatients was not significantly higher than that in isolates from outpatients for all of the antimicrobial/organism combinations except for ceftazidime/*E. coli*. The imipenem-resistant *P. aeruginosa* and ceftazidime-resistant *E. coli* isolates were observed exclusively in isolates from inpatients (Table 4).

Discussion

The study provides insights into the problem of resistance in bacterial Gram-negative enteric pathogens in inpatients and outpatients in YCH, Cameroon. To our knowledge this is the first study in Cameroon determining antimicrobial susceptibility patterns of nosocomial and community-acquired Gram-negative bacilli. Results have demonstrated that in general, Gram-negative bacilli have high rates of resistance to the commonly used antibiotics. A high incidence of resistance was expressed by all bacilli to the penicillins, first generation cephalosporins and trimethoprim/sulfamethoxazole. Many multi-resistant strains were detected. The rates of antimicrobial resistance reported in this study were much higher than those published in developed countries but are similar to those reported in other developing countries.[9,12–17]

Many factors have contributed to such high rates of resistance, including misuse of antibiotics by health professionals, unskilled practitioners and laypersons, misuse of antibiotics by the public (antibiotics can be purchased without prescription), poor drug quality, unhygienic conditions accounting for the spread of resistant bacteria, and inadequate surveillance (lack of information from routine antimicrobial susceptibility testing of bacterial isolates and surveillance testing of bacterial isolates and surveillance of antibiotic resistance, all of which are crucial for good clinical practice and for rational policies against antibiotic resistance).[18]

Our analysis suggests that the rate of resistance in nosocomial pathogens to a variety of antimicrobials, such as amoxicillin, amoxicillin/clavulanate, cefazolin, cefoxitin, cefotaxime and gentamicin, commonly used to treat nosocomial infections, is significantly higher in the hospital setting than the outpatient setting. The high rate of antimicrobial resistance in pathogens isolated in the hospital could possibly be explained by the selective

effect of treatment with multiple antimicrobials for a single patient, which may result in the amplification of antimicrobial resistance in some organisms.[2,19]

This study has demonstrated that the hospital is the focus of antimicrobial resistance. However, there were exceptions. The increasing percentage of trimethoprim/sulfamethozaxole-resistant Enterobacteriaceae; ceftazidime-resistant non-fermenter Gram-negative bacilli; inhibitor-resistant TEM phenotype-resistant Enterobacteriaceae; high level cephalosporinase phenotype-resistant non-fermenter Gram-negative bacilli, and ceftazidime-resistant *E. cloacae* among isolates from outpatients suggest that some form of selection effect on these isolates exists for the outpatients of this hospital.[2]

Some authors have observed in the hospital settings a strong correlation between ceftazidime usage and occurrence of ceftazidime-resistant *P. aeruginosa* and *E. cloacae* isolates.[20–24] In this study, the incidence of ceftazidime-resistant *P. aeruginosa* and *E. clocae* isolates was high. However, information about ceftazidime usage in YCH was not available.

The combination of trimethoprim-sulfamethoxazole is extensively used in Africa owing to its antimicrobial spectrum of activity and its low cost. In addition, resistant bacteria have been isolated from the stool flora of infants who have never been exposed to antibiotics, in contrast to the situation in well developed countries.[25] This reflects the role of poor sanitation in the emergence and dissemination of resistant strains.

The results confirm previously reported findings about the occurrence of high rates of resistance of Gram-negative bacilli to β-lactam antibiotics and trimethoprim/sulfamethoxazole in developing countries, which are much higher than those observed in developed countries. As the incidence of antimicrobial resistance is higher in inpatients' than outpatients' pathogens and because infection control measures may be difficult to implement, more resources should be allocated in the Cameroon to stem possible problems within hospitals, including good antibiotic practices and improvements in hygiene.

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