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Epidemiology of antibiotic resistance in Burkina Faso

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Abstract

Burkina Faso (West Africa) is a tropical country with a high incidence of infectious diseases. The uncontrolled use of antibiotics against bacterial pathogens has given rise to the emergence of antibiotic resistance in this country. The aims of this study were. i) to determine the prevalences of the most important pathogenic bacteria, isolated in the town of Ouagadougou. ii) to identify the bacterial species which have acquired resistance as a result of antibiotic selection. iii) to compare antibiotic-resistances *of Escherichia coli* isolated from stool culture in the present study, with results obtained in 2002 from strains collected in the same structure in Burkina Faso. iv) to determine the trend of antibiotic resistance in Burkina Faso in order to give local advice on the most appropriate empiric antibiotic therapy. Six thousand two hundred and sixty four samples of blood, stools, urine, sputum, pus and vaginal secretion were collected and analyzed in Saint Camille Medical Center (SCMC) laboratory from May 2001 to May 2006. Out of the 6264 samples tested no pathogen was identified in 1583 (25.31%), whilst 4681 (74.73%) were positive, with the incidence of the microrganisms isolated being as follows: *Escherichia coli* 1291 (27.6%), *Staphylococcus aureus* 922 (19.7%), *Salmonella* spp 561 (12.0%), *Streptococcus* spp 499 (10.7%), *Klebsiella* spp 359 (7.7%), *Shigella* spp (6.3%), *Acinetobacter* spp 266 (5.7%) and others 783 (16.7%). Among the isolated pathogens, the highest resistance was found to Amoxycillin: *Proteus* spp 95.6%, *Escherichia coli* 78.2%, *Salmonella spp* 62.2%, *Shigella* spp 73.4% and *Klebsiella* spp 89.9%, followed by resistance to Ampicillin and cotrimoxazole. Comparing the prevalence of antibiotic resistance of *Escherichia coli* from stool cultures isolated during 1999-2000 to that of 2001-2006, a significant reduction was found, which could be due to the improved use of antibiotics in recent years. The reduced antibiotic-resistance observed in pathogens isolated in Burkina Faso during this study as compared to previous data, could be the result of setting up microbiological epidemiological monitoring centres, in tropical countries, to better control the emergence of bacterial antibiotic-resistance.

Keywords: *Escherichia coli*; antibiotic-resistance; Burkina Faso

INTRODUCTION

Worldwide the emergence of multiresistant bacteria (BMR), able to survive treatment with several classes of antibiotics, has given rise to reduced or absent therapeutic options for the treatment of infectious diseases [1,2]. Previous irrational use of antibiotics with broad spectrum activity, like the second and third generation cephalosporins, has lead to resistance to methicillin, even in infected individuals who had never been exposed to methicillin [3]. Other causes such as incorrect diagnoses, abusive prescrip-

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tions, inappropriate use of antibiotics by patients, self medication, excessive drug consumption and the use of antibiotics as food supplements in veterinary medicine has also contributed to antibiotic resistances[4].

In fact in many developing countries like Burkina Faso, resistance to antibiotics has become a major public health concern with economic and social implications. In this country the availability of the different antibiotics is limited and usually betalactams, aminoglycosides and quinolones are used for the treatment of infections.

The aim of this study was: i) to determine the prevalences of the most important pathogenic bacteria in the town of Ouagadougou; ii) to identify the bacteria that have acquired antibiotic-resistance under the selective antibiotic pressure in Burkina Faso; iii) to compare resistances of *Escherichia coli* isolated from stool cultures in 2002 by Bonfiglio et al (2002)[5] in Burkina Faso with our present results and lastly; iv) to draw conclusions from our study in order to give advice that will enable to reduce further the bacterial resistances to antibiotics usually utilized in Burkina Faso.

MATERIALS AND METHODS

Sampling and equipment

From May 2001 to May 2006, 6264 samples of blood, stools, urine, sputum, pus and vaginal secretion were collected from outpatients and tested in Saint Camille Medical Center (SCMC) laboratory. Each sample was inoculated and incubated in aerobic conditions at 37° C or according to specific requirements in an anaerobic environment. In this study, 1335 stool cultures, 1878 urine cultures, 1180 cultures of vaginal swabs, 245 of pus and 43 of other clinical specimens such as blood, sputum and cerebrospinal fluid (CRL) were carried out. After being isolated and identified, the bacteria were exposed to various antibiotics concentrations, in order to determine the minimal inhibitory concentrations (MICs), according to the method described by the Clinical and National Committee for Clinical and Laboratory Standards (NCCLS), 1998(6)[6].

Identification

The bacterial identification was carried out on the basis of morphological characteristics by observing the colonies and confirming the results by Api 20 system (BioMerieux, France) for enterobacters and by Api 20 Staph and Api 20 Strep for *staphilococcus* spp and *streptococcus* spp respectively.

Antibiotics

Most antibiotics used as substrates in our study, were acquired by Sigma (Chemical Co St Louis, MO, U. S. A) and the abbreviation are summarized as follow: Amoxycillin (Amx), Amoxycillin/clavulanic acid (Amc), Ampicillin (Am), Cefazoline (Cz), Chloramphenicol (Cl), Ciprofloxacin (Cipro), Cotrimoxazole (Sxt), Gentamicin (Gm), Nalidix acid (Na), Lincomycin (L), Norfloxacin (Nor), Netilmicin (Net), Oleandomicin (Ol), Pristamicine (Pr), Tetracycline (Te).

Antibiotic susceptibility testing

All the strains isolated were tested in order to determine their susceptibility to the various antibiotics through the disc-diffusion method by using agar of Mueller Hinton according to the method recommended by the the National Committee for Clinical and Laboratory Standards (NCCLS), 1998. The agar plates containing the discs were incubated at 37°C for 18-24 hours. After this incubation, the zones of inhibition around the antibiotic discs were measured by means of a calliper.

The multi-resistance to antibiotics was defined as a condition where a specific bacteria is resistent to several antibiotics.

The Ethical Committee of the SCMC approved this study and informed consent was obtained from all patients before collecting the clinical sample for the use of personal data.

Statistical analysis

The prevalence of bacterial isolation and the drug-resistance were recorded on a computer file and analyzed by standard software SPSS 12 (SPSS Inc, USA) for Windows. Statistical significance was set at $P < 0.05$.

RESULTS

1583/6264 (25.27%) cultures of these clinical samples gave negative results and 4681/6264 (74.73%) were culture positive, of these 1421 were shown to be gram-positive bacteria and 3260

gram-negative bacteria. From the 1335 stool cultures carried out in this study the following micro-organisms were isolated: *Salmonella* spp 555 (41.6%), *Shigella* spp 295 (22.1%), *Escherichia coli* 281 (21.1%), *Edwardsiella tarda* 69 (5.2%), *Yersinia* spp 58(4.3%) and 77 other bacteria (5.7%). Other bacterial pathogens and their prevalences, isolated from 1878 urocultures, 1180 vaginal swabs and 245 samples of pus are reported in Table I. In some samples more than one bacterial species were isolated.

The antibiogrammes showed resistances to several antimicrobial agents: the resistance to Amoxycillin of *Acinetobacter* spp, *Escherichia coli*, *Enterobacter* spp, *Proteus* spp, *Salmonella* spp, *Shigella* spp and *Klebsiella* spp were respectively: 44.1%, 78.2%, 70.2%, 95.6%, 6.2%, 73.2 and 89.9%, respectively while the resistance to Ampicillin of *Acinetobacter* spp, *Escherichia coli*, *Enterobacter* spp, *Proteus* spp, *Salmonella* spp, *Shigella* spp and *Klebsiella* spp were: 46.9%, 77.4%, 67.3%, 86.8%, 41.9%, 61.8% and 89.9%, respectively (Table II).

Gram-negative bacteria for the first time appear to be also multi-resistant: in Burkina Faso *Proteus* spp has recently acquired multi-resistances to Ampicillin (86.8%), Amoxycillin (95.6%) and Amoxycillin/clavulanic acid (94.3%). We have also found a high prevalence of *Escherichia coli* resistant to Ampicillin (77.4%), Amoxycillin/clavulanic acid (50.6%), Amoxycillin (78.2%) and Cotrimoxazole (71.2%). *Klebsiella* spp has also acquired multi-resistances to Ampicillin (89.9%), Amoxicillin (89.9%) and Amoxycillin/clavulanic acid (42.7%) (Table II). The resistance to the other antibiotics tested (Cipro, Nor and Na) was very low (< 21%) both for *Escherichia coli* and *Klebsiella*.

Gram-positive bacteria isolated in this study like *Streptococcus* spp and *Staphylococcus* spp have shown a high prevalence of resistance to Lincomycin (82.5% and 54.6% respectively); to Oleandomycin (71.3% and 55.2% respectively) and a very low resistance (1.3% and 0.8% respectively) to Netilmicin (Table III).

Table IV shows the different resistance rates of *Escherichia coli* strains isolated in urine culture, stool culture and genital swab. The differences in the percentages of resistance were significantly only for SXT, Chloramphenicol and Ciprofloxacin ($P < 0.001$).

Lastly, in Table V the percentage of antibiotic-resistance of *Escherichia coli* isolated from stool cultures found in this study shows a significant reduction for Ampicillin and Amoxycillin as compared to that found in 1999-2000 (Bonfiglio et al 2002)[5], while the resistance of Chloramphenicol increased from 4.84% to 32.7%.

Table I: Frequency of pathogenic bacterial isolates from different specimen types at SCMC

	Coproculture (%)	Uroculture (%)	Genital Swabs (%)	Pus (%)	Other (%)	Overal (%)
Escherichia coli	281(21.1)	611(32.5)	360(30.5)	39 (15.9)	0(0.0)	1291(27.6)
Salmonella spp.	555(41.6)	6(0.3)	0(0.0)	0(0.0)	0(0.0)	561 (12.0)
Shigella spp.	295(22.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	295(6.3)
Acinetobacter	6(0.5)	195 (10.4)	59 (5.0)	6 (2.4)	0 (0.0)	266 (5.7)
Enterobacter spp.	11 (0.8)	101 (5.4)	67 (5.7)	0 (0.0)	0 (0.0)	179 (3.8)
Haemophilus spp.	0 (0.0)	0 (0.0)	11 (0.9)	0 (0.0)	0 (0.0)	11 (0.2)
Proteus spp.	30 (2.3)	34 (1.8)	6 (0.0)	65 (26.5)	6 (13.9)	141 (3.0)
Pseudomonas spp.	0 (0.0)	0 (0.0)	0 (0.0)	12 (4.9)	0 (0.0)	12 (0.3)
Streptococcus spp	0 (0.0)	156 (8.3)	292 (24.7)	34 (13.9)	17(39.5)	499 (10.7)
Yersinia spp.	58 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	58 (1.2)
Edwardsiella tarda	69 (5.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	69 (1.5)
Klebsiella spp.	12 (0.9)	184 (9.8)	147 (12.5)	12 (4.9)	10(23.2)	359 (7.7)
Pleiomonas shighelloides	18 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	18 (0.4)
Staphylococcus aureus	0 (0.0)	591 (31.5)	238 (20.2)	77 (31.4)	16(37.2)	922 (19.7)
	1335(100.0)	1878(100.0)	1180(100.0)	245(100.0)	43(100.0)	4681 (100.0)

Table II : Percentage of Gram-negative bacterial isolates resistant to antimicrobic agents

Drug	*Acinetobacter*	*Escherichia coli*	*Enterobacter* spp.	*Proteus* spp.	*Salmonella* spp.	*Shigella* spp.	*Klebsiella* spp.
Am	(46.9%) 122/260	(77.4%) 943/1218	(67.3%) 113/168	(86.8%) 118/136	(41.9%) 233/556	(61.7%) 182/295	89.9%) 303/337
Amx	(41.9%) 109/260	(78.2%) 953/1218	(70.2%) 118/168	95.6%) 130/136	(62.2%) 346/556	(73.2%) 216/295	(89.9%) 303/337
Amc	(40.8%) 106/260	(50.6%) 616/1218	(46.4%) 78/168	(94.1%) 128/136	(48.4%) 269/556	(20.7%) 61/295	(42.7%) 144/337
Gm	(8.5%) 22/260	(9.8%) 119/1218	(0.0) 0/168	(5.1%) 7/136	(0.0) 0/556	–	(3.6%) 12/337
Net	(4.2%) 11/260	(4.6%) 56/1218	(3.6%) 6/168	(6.6%) 9/136	(0.0) 0/556	(0.0) 0/295	(5.9%) 20/337
Sxt	(50.4%) 131/260	(71.2%) 867/1218	(24.4%) 41/168	(38.2%) 52/136	(23.8%) 132/556	(45.8%) 135/295	(40.6%) 137/337
Cl	–	(17.1%) 208/1218	–	–	(9.7%) 54/556	(21.7%) 64/295	–
Te	–	(66.1%) 805/1218	–	–	(32.7%) 182/556	(70.2 %) 207/295	–
Na	(16.7%) 42/260	(20.9%) 254/1218	(16.7%) 28/168	(37.5%) 51/136	(12.0%) 67/556	(15.2%) 45/295	(12.7%) 43/337
C	(30.4%) 79/260	(21.7%) 264/1218	(23.2%) 39/168	(24.3%) 37/136	(10.1%) 56/556	(12.2%) 36/295	(28.5%) 96/337
Cipro	(6.5%) 17/260	(13.0 %) 159/1218	(0.0) 0/168	(0.0) 0/136	(0.0) 0/556	(8.8 %) 26/295	(0.0) 0/337
Nor	(12.2%) 31/260	(4.8%) 58/1218	(0.0) 0/168	(0.0) 0/136	(0.0) 0/556	(2.7%) 8/295	(2.1%) 7/337

Table III : Percentage of Gram-positive bacterial isolates resistant to antimicrobic agents.

Drug	*Streptococcus* spp.	*Staphylococcus* spp.
Am	(23.0%)104/453	(51.4%)457/888
Amx	(40.6%)184/453	(56.4%)501/888
Amc	(20.7%)94/453	(44.3%)393/888
Gm	(25.4%)115/453	(2.5%)22/888
Net	(1.3%)6/453	(0.8%)7/888
Cz	(42.1%)191/453	(24.8%)220/888
Ol	(71.3%)323/453	(55.2%)490/888
L	(82.5%)374/453	(54.6%)485/888
Pr	(36.4%)165/453	(43.2%)392/888

Table IV : Percentage of *Escherichia coli* isolates from different specimen resistant to antimicrobial agents

Drug	*Escherichia coli*							
	Sxt	Am	Amx	Amc	Cl	Nor	Na	Cipro
Stool	237/281	202/281	213/281	108/281	89/281	32/281	57/281	16.5/281
Culture	83.3%	71.8%	75.8%	38.5%	31.6%	11.3%	20.2%	5.9%
Urine	447/611	502/611	483/611	315/611	121/611	29/611	130/611	133/611
Culture	73.1% *	82.1%	79.0%	49.5%	19.8% *	4.7%	21.3%	21.7% *
Genital	259/360	291/360	288/360	183/360	72/360	17/360	79/360	75/360
Culture	71.9% *	80.8%	80.1%	61.8	20% *	4.7%	21.9%	20.8% *

Stool→ Urine→Genital * $P<0.001$

Table V: Comparison of resistance of isolated *Escherichia coli* in coproculture by Bonfiglio et al. 2002 and Simpore et al. 2006

Drug	Coproculture : *Escherichia coli*				*P*
	Bonfiglio et al 2002		Simpore et al 2006		
	N°	%	N°	%	
Am	274/289	94.8	216/281	76.8	<0.01
Amx	274/289	94.8	232/281	82.6	<0.01
Amc	136/289	47.0	123/281	43.7	0.465
C	14/289	4.84	92/281	32.7	<0.01
Sxt	231/289	79.9	234/281	83.3	0.303
Nor	40/289	13.8	32/281	11.4	0.654

DISCUSSION

In the world, infectious diseases account for 17 million of deaths per year, which represents one third of the mortality in the world [7]. They represent 43 % of the deaths in developing countries against 1 % of those in industrialized countries. This situation is likely to worsen as a result of the fast worldwide emergence of antibiotic multiresistant microbial strains.

Among the 6264 blood, stools, urine, sputum, skin, pus and vaginal swab clinical samples collected and tested in SCMC laboratory, 4681 were positive for bacterial culture (74.73%). A high frequency of isolation of *Escherichia. coli* (32.5%) was found from urine cultures. This prevalence is almost equal to that found by Lin et al 2006 (29.7%) in institutionalized elderly living in Taiwan [8] and by Bonfiglio et al 2002 (35.0%) in Burkina Faso [5], but definitely lower than those identified by Abdullah et al. 2005 (66.7%) [9] in United Arab Emirates, Al-Haddad AM 2005 (41.5%) [10] and Mohanna and Rajaá 2005 (66.3%) [11] in Yemen, probably due to different identification method. The results of the antibiotic susceptibility of *Escherichia coli* detected by Mohanna and Rajaá, 2005 [11] differed from ours respectively: Nalidixic acid (70.0% and 79.1%); Amoxycillin/clavulanic acid (29.9% and 49.4%) and Cotrimoxazole (16.4% and 22.6%). We also identified 9.8% of *Klebsiella* spp. This percentage is lower than that found by Lin et al, 2006 (21.6%) [8] in China, but higher than that of Mohanna and al, 2005 (3.9%) [11]. *Staphylococcus* spp (19.7%), *Salmonella* spp (12.0%), *Streptococcus* spp (10.7%), *Klebsiella* spp (7.7%) and *Acinetobacter* spp (5.7%) were also isolated in a variable percentage of cases according the different type of clinical specimens (Table I).

In the case of the Gram-positive bacteria isolated in our study, *Streptococcus* spp and *Staphylococcus* spp have shown respectively low resistance rates to Netilmicin (1.3% and 0.8%), respectively While high resistance rates of these bacterial species were found to Lincomycin (82.5 % and 54.6 %) and Oleandomycin (71.3% and 55.2%) respectively (Table III).

The issues of resistances to antimicrobials are a worldwide phenomenon. In tropical developing countries where there are many infectious diseases and few available antimicrobial drugs, an alarm bell was given by the work of Bonfiglio et al. in 2002 [5], which showed for the first time a high resistance to Ampicillin and Amoxycillin in Burkina Faso. Since then, clinicians have prescribed less frequently these antibiotics. When we compare the results of the previous study by Bonfiglio et al carried out in the peri-

od 1999-2000 and published in 2002 [5] on antimicrobial resistance (to Ampicillin and Amoxycillin) in Burkina Faso to those of this study, carried out between 2001 and 2006 we demonstrate statistically significant differences ($P < 0.01$) (Table V). We did not find statistically significant differences as far as the variation of resistance to Amc, Sxt and Nor is concerned. While chloramphenicol seems to have now acquired an alarming level of resistance in Burkina Faso. Its resistance has in fact increased from 5% in 1999-2000 period to > 32% in 2001-2006 ($P < 0.01$).

With regards to nosocomial infections, the levels of resistance of *Streptococcus* spp to beta-lactam in this study were found to be 20-40%. Surveys of the EARSS (European Antimicrobial Resistance Surveillance System) have reported resistance of 53% in France, 50% in Romania, 33% in Spain and 30% in Poland [12].

We have not data about resistance of *Streptococcus pyogenes* to macrolides as is now frequently observed in Europe[13] and the only observation which we may be refered is the resistance of *Streptococcus* spp to Oleandomycin.

In many developing countries lacking bacteriology laboratories, it is not possible to rely on microbiological cultures and on the results of an antibiogramm so physicians and nurses prescribe antibiotics only on the basis of the patient's clinical signs and on their own experiences .

In nations where bacteria have become extremely resistant to Amoxycillin, Ampicillin and Chloramphenicol, it may be sufficient to interrupt the prescription of these drugs and to use other new molecules (quinolones) so as to regain bacterial susceptibility in the absence of pharmacological pressure. The results of this study demonstrate at least for the bacteria studied that this has been possible in Burkina Faso.

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