Epidemiology of bacterial resistance in gastro-intestinal pathogens in a tropical area

Giovanni Bonfiglio [a], Jacques Simporè [b], Salvatore Pignatelli [b], Salvatore Musumeci [c,d,*], Maria Letizia Solinas [c,d]

[a] *Dipartimento di Scienze Microbiologiche, University of Catania, Catania, Italy*
[b] *Centre Medical St. Camille, Ouagadougou, Burkina Faso*
[c] *Department of Pediatrics, Cattedra di Pediatria Sociale e Puericultura, University of Sassari, Viale San Pietro 12, 07100 Sassari, Italy*
[d] *Institute of Population Genetics, Italian National Research Council, Alghero, SS, Italy*

Abstract

During 1999–2000 a total of 4131 faecal specimens were collected and analysed at the medical centre St. Camille at Ouagadougou. Eight hundred and twenty-six (8.0%) grew significant bacteria. *Escherichia coli* (35%), *Salmonella* spp. (15%) and *Shigella* spp. (10%) were most frequently isolated. A large number of *E. coli* strains were resistant to aminopenicillins (> 90%) and cotrimoxazole (80%); for *Yersinia* spp the resistance was 80 and 25%, respectively. Norfloxacin was the most active antibiotic but was rarely used. The study showed that it is necessary to create antibiotic-resistance surveillance centres in developing countries so that therapy may be appropriate and the spread of antibiotic resistance to other developed countries via increased emigration may be reduced.

1. Introduction

Burkina Faso is a western African nation of 274 000 km^2 and with 12 000 000 citizen of whom 10% live in the capital Ouagadougou. Burkina Faso is one of the poorest African nations and the economy is based on agriculture. The health system comprises two national and nine regional hospitals. There is no national means of monitoring antibiotic resistance.

One of the most important problems of this nation is the high level of starvation, in particular in the children, due principally to poor diet and dietary taboos especially in those groups belonging to strict religions. In addition, the children have frequent intestinal infections including parasitic infestations and bacterial diarrhoea that carry a very high mortality [1,2]. Bacterial diarrhoea diseases are frequent and the microorganisms responsible are usually unknown and in many cases are responsible of the death of these patients because of therapy failure [3].

The aim of this study was to look at bacteria responsible for episodes of diarrhoeal disease in patients at the medical centre of St. Camille at Ouagadougou during 1999–2000. The antibiotic susceptibility of the isolated microorganisms was also studied.

2. Materials and methods

2.1. Collection of material

During 1999–2000, a total of 4131 faecal specimens were collected from different patients. Microscopic examination was performed on every specimen to determine the presence of leukocytes, erythrocytes and mucous.

Faecal material was cultured on Hektoen enteric, Salmonella Shigella and MacConkey agar and incu-

* Corresponding author. Tel.: +39-360-285505; fax: +39-095-7179690

E-mail address: smusumeci@tiscalinet.it (S. Musumeci).

bated for at least 48 h at 37 °C; plates were examined daily. MacConkey sorbitol agar was used for enterotoxic *Escherichia coli* strains 0–157, cefsulodin igarsannovobiocin (CIN) agar for *Yersinia* spp isolation, and Campy BAP incubated in a CO_2 atmosphere for Campylobacter isolation.

2.1.1. Identification

Bacterial identification was carried out on the basis of morphological characteristics and colony observation and was confirmed using the API 20 system (BioMerieux).

2.1.2. Susceptibility tests

All isolated strains were tested for their susceptibility to different antibiotics using an agar diffusion method on Mueller Hinton agar according to the methodology recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [4].

The antibiotics tested were ampicillin, amoxycillin, amoxycillin/clavulanic acid, cotrimoxazole, tetracycline, colistin, chloramphenicol and norfloxacin. Antibiotic breakpoints defined by NCCLS [5] were used.

Inoculated plates containing discs were incubated at 35 °C for 18–24 h after which the inhibition zones around the antibiotic discs were measured using a calliper.

3. Results

During the 2-year study a total of 4131 faecal specimens were examined. Three hundred and thirty (8%) were positive for protozoa, principally *Giardia* spp., *Entamoeba histolytica* and *Trichomonas hominis*. In a few specimens *Blastocystis hominis*, *Balantidium coli* and helminth eggs were found. *Candida* spp. was seen in almost half of the specimens. In 2975 specimens (72.0%), microscopical examination was negative for protozoa, worms, leucocytes and erythrocytes and a viral aetiology was suspected. Bacterial isolation was attempted for 826 specimens (20.0%) in which the microscopic examination showed the presence of leucocytes and blood.

Of the 826 positive specimens, 8.0% grew the organisms shown in Table 1. The most common isolate was *E. coli* (35%) followed by *Salmonella* spp. (15%) and *Shigella* spp. (10%).

Table 2 shows the percentage antibiotic susceptibility of the most commonly isolated microrganisms. Only 4% of *E. coli* strains were susceptible to ampicillin and amoxycillin and 50% to amoxycillin/clavulate. Resistance to cotrimoxazole was 80% but relatively few strains were resistant to colistin and norfloxacin.

Twenty-two (22.0%) of *Salmonella paratyphi* strains were susceptible to amoxycillin and almost 75% to

Table 1
Percentage of different microorganisms isolated during the study (1999–2000)

Microorganisms	Total isolates (%)
E. coli	34
S. paratyphi	14
Klebsiella pneumoniae	14
Shigella flexneri	10
Klebsiella oxytoca	6
Edwarsiellae	6
Yersinia enterocolitica	4
Citrobacter spp.	4
Proteus spp.	3
Providencia spp.	3
Salmonella typhi	2

Table 2
Antibiotic susceptibility of the most common bacteria

Antibiotics	Susceptibility (%)			
	E. coli	*Y. enterocolitica*	*S. flexneri*	*S. paratyphi*
Ampicillin	5	20	28	26
Amoxycillin	5	22	36	26
Clindamicin	95	22	91	94
Amox/clav	53	33	60	41
Tetracycline	nt	22	35	84
Cotrimoxazole	20	44	55	78
Colistin	22	64	85	92
Norfloxacin	86	100	100	97

nt, Not tested.

cotrimoxazole; colistin and norfloxacin were most active.

Similarly, resistance of *Yersinia* spp. was high to the aminopenicillins but low to norfloxacin and colistin. Norfloxacin was by far the most active antibiotic against organisms tested (Table 2).

Shigella spp were also highly resistant to aminopenicillins and highly susceptibility to norfloxacin and clindamicin; 25% of strains were sensitive to amoxycillin/clavulanate.

Over the 2 years of study (1999–2000) *Salmonella* spp. became more resistant to the aminopenicillins and resistance to amoxycillin/clavulanate increased in both *Salmonella* spp and *Shigella* spp.

The decreased use of cotrimoxazole was paralleled by an increase in susceptibility to this antibiotic. No significant change of susceptibility of *E. coli* was observed during the 2 years of the study.

We also analysed the antibiotic susceptibility of microorganisms isolated from other patients living 100 km from the capital (Koupela and Nanorò). The percentage of protozoa was very similar (9%) and both the percentage of pathological microorganisms isolated and their antibiotic resistance was similar to those isolated in the capital Ouagadougou.

4. Discussion

Every year more than 3 000 000 children die of diarrhoeal diseases [6]. Rotavirus infections are more frequent in children, whereas bacterial infections are more frequent in adults or the young [2]. The most common microorganisms responsible for intestinal disease are *E. coli*, *Shigella* spp., *Salmonella* spp., *Yersinia* spp. and very rarely *Vibrio cholerae* existing in well defined areas [7]. In Burkina Faso, laboratory support is available in only a few hospitals and many patients are cured empirically by the use of minerals and rehydration distributed by the WHO; often oral antibiotics are used, especially cotrimoxazole [8]. The incorrect use of antibiotics has resulted in a very high incidence of resistance. Infection with *Salmonella* spp. is responsible for more than 700 000 deaths per year [9]. This has been treated with chloramphenicol, ampicillin or cotrimoxazole, but recently several multiresistant *Salmonella* spp. strains have been isolated [10]. Aminopenicillins, cotrimoxazole and nalidixic acid are widely used to treat infections with *Shigella* spp and this has lead to resistance development. The high susceptibility to expensive antibiotics rarely used in this country such as norfloxacin is further confirmation of the selective pressure resulting from the incorrect use of antibiotics. Such antibiotics should only be used for limited periods, preferably at high dosage to conserve their activity and avoid the development of resistance. In Tajikistan outbreaks with *S. typhi* resistant to ciprofloxacin are now common [11].

The similarities in resistance between Ouagadougou and the two distant villages further confirms general antibiotic misuse.

The faecal flora is recognised as a reservoir of resistance genes both in the developed and developing countries [12,13]. Misuse of antibiotics in developing countries could result in spread to other countries and an increase of the surveillance units in these developing countries is important not only to address to the problems of improving the economic status but also to fight and control infectious diseases.

General strategies for the development of a controlled policy could be suggested (i) adoption of guidelines for the correct use of antibiotics to avoid the development and selection of resistant microorganisms (ii) to improve the hygiene in the population to prevent spread of antibiotic resistance and (iii) monitoring antibiotic resistance of microorganisms locally, nationally and internationally to contain widespread resistance. Where possible a rotation of therapy could be used by stopping the use of an antibiotic when its resistance increases. This has been shown to be effective in reducing resistance to that antimicrobial and eliminating both an outbreak and the clone of resistant bacteria involved [14–16].

Only if these suggestions are adopted shall we be able to prevent the spread of antibiotic resistance.

References

[1] Murray CJL, Lopes AD. Mortality by cause for eight regions of the world: global burden of disease study. Lancet 1997;349:1269–76.

[2] Stephen J. Pathogenesis of infectious diarrhea. Can J Gastroenterol 2001;15:669–83.

[3] Hart CA, Kariuki S. Antibiotic resistance in developing countries. Br Med J 1998;317:647–50.

[4] National Committee for Clinical Laboratory Standards. Performance Standards for antimicrobial disk susceptibility tests, sixth ed. Approved Standards, M2-A6, NCCLS, Wayne, PA, 1997.

[5] National Committee for Clinical Laboratory Standards. National Committee for Clinical Laboratory Standards: Performance standards for antimicrobial susceptibility testing; eighth informational supplement, M100-S8. NCCLS, Wayne, PA, 1998.

[6] Hart CA, Cunliffe NA. Viral gastroenteritis. Curr Opin Infect Dis 1997;10:408–13.

[7] Hossain MA, Rahaman M, Ahmed QS, Malek MA, Sack RB, Albert MJ. Increasing frequency of mecillinam-resistant shigella isolates in urban Dhaka and rural Matlab, Bangladesh: a 6 year observation. J Antimicrob Chemother 1998;42:99–102.

[8] Parkes G. Treating diarrhoea. Rehydratation should have been emphasised. Br Med J 1997;315:1378.

[9] Green SDR, Cheesbrought JS. Salmonella bacteraemia among young children at a rural hospital in western Zaire. Ann Trop Paediatr 1993;13:45–53.

[10] Mirza SH, Beeching NJ, Hart CA. Multidrug resistant typhoid: a global problem. J Med Microbiol 1996;44:317–9.

[11] Murdoch DA, Banatvala N, Bone A, Shoismatulloev B, Ward LR, Threlfall EJ. Epidemic ciprofloxacin-resistant *Salmonella typhi* in Tajikistan. Lancet 1998;351:339.

[12] Shanahan PMA, Thomson CJ, Amyes SGB. Beta-lactam resistance in aerobic faecal flora from general practice patients in the UK. Eur J Clin Microbiol Infect Dis 1994;13:760–3.

[13] Shanahan PMA, Wylie BA, Adrian PV, Koornhof HJ, Thomson CJ, Amyes SGB. The prevalence of antimicrobial resistance in human faecal flora in South Africa. Epidem Infect 1993;111:221–8.

[14] Pear SM, Williamson TH, Bettin KM, Gerding DN, Galgiani JN. Decrease in nosocomial *Clostridium difficile*-associated diarrhea by restricting clindamycin use. Ann Intern Med 1994;120:272–7.

[15] Johnson S, Samore MH, Farrow KA, et al. Epidemics of diarrhea caused by a clindamycin-resistant strain of *Clostridium difficile* in four hospitals. New Engl J Med 1999;341:1645–51.

[16] John JF, Jr, Rice LB. The microbial genetics of antibiotic cycling. Infect Control Hosp Epidemiol 2000;21(Suppl):S22–31.