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Evaluation of a rapid screening method for detection of antimicrobial resistance in the commensal microbiota of the gut

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Summary The assessment of antimicrobial resistance among commensal bacteria is an indicator of the spread of antimicrobial resistance. Rapid screening methods for detection of antimicrobial-resistant faecal *Escherichia coli* directly on MacConkey plates have been successfully adopted but suffer from lack of standardisation. The purpose of this study was to evaluate a direct plating method (DPM) for detection of antimicrobial-resistant faecal *E. coli* and to compare it with a conventional method. Faecal samples were collected from 71 healthy children from Peru and Bolivia.

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In the DPM, a faecal swab was directly plated onto a MacConkey agar plate and antimicrobial disks were applied onto the seeded plate. Raw data were obtained by direct reading of the plate and were subjected to confirmatory analysis. Good concordance between the DPM and a conventional method was observed in detecting carriage of resistant *E. coli*, with a higher sensitivity for the DPM. Analysis of the results allowed interpretive criteria to be defined for DPM raw data. The DPM showed good sensitivity and specificity at very low cost (ten times cheaper than the conventional method) to investigate the faecal carriage of drug-resistant *E. coli*. It may represent a useful tool to conduct large-scale resistance surveillance studies and to monitor resistance control programmes cost effectively, particularly in low-resource countries.

1. Introduction

Bacterial resistance to antimicrobial agents represents a global public health problem. It is particularly serious in low-resource countries where bacterial infections remain among the major causes of death, especially in childhood (WHO, 2001).

Surveillance of antimicrobial susceptibility is a key element to provide updated information on the magnitude and trends in resistance, and to plan and monitor intervention strategies aimed at preserving the therapeutic efficacy of antimicrobial agents. Surveillance programmes sponsored by multinational organisations (e.g. Network on Antimicrobial Resistance, WHONET) or pharmaceutical companies (e.g. SENTRY, Bristol-Myers Squibb) are currently active in several countries. These programmes focus their attention on antimicrobial resistance in human clinical isolates and are based on the acquisition of data from selected laboratories using standardised and controlled techniques for sensitivity testing and interpretation. In low-resource countries, effective surveillance programmes are difficult to implement for a number of reasons, including scarce financial resources, lack of laboratory facilities and, where laboratories do exist, lack of quality control, reliable reagents and adequate supervision (Shears, 2001). The limited information based on reliable data on antimicrobial resistance for low-resource countries is mostly from studies conducted on pathogens isolated during disease outbreaks or from community- or hospital-acquired infections observed in the few health centres where high-quality laboratories are available (Guzmán-Blanco et al., 2000; Hart and Kariuki, 1998; Rodríguez et al., 2001).

There is an increasing agreement about the importance of extending the surveillance of antimicrobial resistance to the commensal microbiota of humans and animals (ROAR; http://www.tufts.edu/med/apua/ROAR/project.htm). This bacterial population, although not being a specific target, is continuously exposed to the selective pressure generated by antimicrobial chemotherapy and may become a potential reservoir of resistant strains that can cause infections, and of resistance determinants that can be transferred to pathogenic bacteria (Kariuki and Hart, 2001). Therefore, surveillance of antimicrobial-resistant bacteria carried by healthy individuals is considered an indicator of the spread of antimicrobial resistance that could also be useful to predict the emergence of resistance in pathogenic bacteria (Calva et al., 1996; Levin et al., 1997). In this perspective, resistance patterns of some members of the commensal microbiota, such as the faecal *Escherichia coli*, have been evaluated in various epidemiological settings (Bartoloni et al., 1998, 2004; Calva et al., 1996; Datta, 1969; Lester et al., 1990; Linton et al., 1972; Moorhouse, 1969; Okeke et al., 2000; Österblad et al., 2000; Shanahan et al., 1993).

Different approaches have been used to evaluate the faecal carriage of resistant *E. coli*, including: (a) conventional methods based on testing antimicrobial susceptibility in a small number (5—10) of randomly selected colonies of *E. coli* isolated from faecal samples plated on selective media for *Enterobacteriaceae* (Lester et al., 1990; Okeke et al., 2000; Österblad et al., 2000); and (b) methods based on direct plating of faecal samples, or dilutions thereof, on selective media for Enterobacteriaceae, in the presence of antimicrobial agents, either applied on disks or incorporated in the media (Bartoloni et al., 1998, 2004; Calva et al., 1996; Datta, 1969; Lester et al., 1990; Linton et al., 1972; Moorhouse, 1969; Shanahan et al., 1993). In principle, the latter methods are preferable owing to their higher sensitivity (Lester et al., 1990), as well as being faster and less expensive, but methodological standardisation is currently lacking.

The purpose of this study was to evaluate the performance of a direct plating method (DPM) for

detection of antimicrobial resistance of faecal *E. coli* from healthy subjects and to compare the results with those obtained with a conventional method.

2. Materials and methods

2.1. Samples

The study was carried out on samples obtained from 71 healthy children aged 6—72 months from urban and rural areas of Peru (Moyobamba, $N = 15$; Yurimaguas, $N = 9$; San Juan de Candado, $N = 7$) and Bolivia (Camiri, $N = 14$; Villa Montes, $N = 18$; Alto Ipoguatzu, $N = 8$). The samples were collected during the pilot study of the ANTRES project, a co-operative research project financed by the European Community aimed at describing antimicrobial use and resistance in low-resource countries (http://www.unifi.it/infdis/antres/default.htm).

Written informed consent was obtained from parents or authorised legal guardians of minors. Ethical approval for this study was provided by the ethical committees of the participating institutions. A rectal swab was obtained from each subject, preserved in Amies transport medium (Oxoid, Basingstoke, UK) and transferred in a cold box to the laboratories within 3 h after collection.

2.2. DPM and conventional method

The DPM for detection of antimicrobial-resistant *E. coli* was carried out essentially as described previously (Bartoloni et al., 2004). Each swab was plated onto a MacConkey Agar No. 3 plate (Oxoid) to obtain uniform growth, and antimicrobial disks were immediately applied onto the seeded plates. The following antimicrobial agents (disk potency) were tested: ampicillin (10 μg); cefotaxime (30 μg); sulphamethoxazole/trimethoprim (23.75/1.25 μg); nitrofurantoin (300 μg); nalidixic acid (30 μg); ciprofloxacin (5 μg); tetracycline (30 μg); chloramphenicol (30 μg); amikacin (30 μg); gentamicin (10 μg); and tobramycin (10 μg) (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). After 12—14 h incubation at 37 °C, the plates were inspected for growth. Only samples yielding confluent or subconfluent colonies with a morphological appearance typical of *E. coli* were included in the study. One of the following situations could be observed around each disk: (i) presence of a growth inhibition zone; (ii) absence of a growth inhibition zone; and (iii) presence of a growth inhibition zone with colonies growing inside

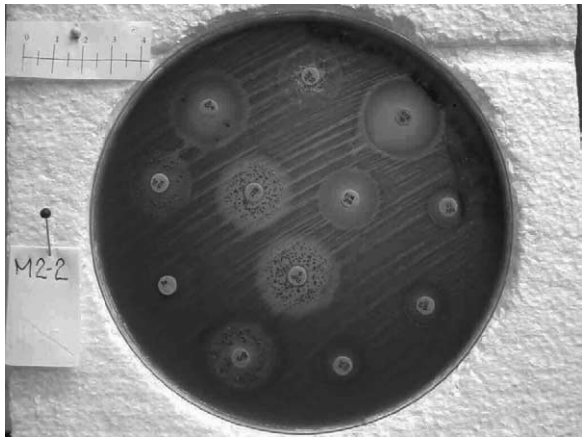

Figure 1 MacConkey agar plate yielding uniform coliform growth in the direct plating method after approximately 14 h of incubation at 37 °C. Inhibition zones are observed around some antimicrobial-containing disks, with internal colonies present in some cases. No inhibition zone is observed around other disks. (The photograph shows a plate with 12 disks; in the present study only 11 disks were used.)

(with colonial morphology that may or may not be coliform) (Figure 1). Inhibition zone diameters were measured. In cases (i) and (ii) described above, a loopful of the microbial growth at the edge of the inhibition zone or in the proximity of the disk edge was collected, whilst in case (iii) a pool of the coliform colonies growing inside the inhibition zone was collected. Collected bacteria were preserved in cystine—trypticase agar tubes (Becton Dickinson) to undergo confirmatory analysis. For this purpose, the preserved cultures were isolated on MacConkey agar and lactose-fermenting colonies with a morphological appearance of *E. coli* were identified using the API 20E identification system (BioMérieux, Marcy l'Étoile, France). The confirmed *E. coli* isolates were then tested for antimicrobial susceptibility to the corresponding panel of antimicrobials by standard disk diffusion method.

In the conventional method, the same swab used to perform the DPM was inoculated onto a MacConkey Agar No. 3 plate (Oxoid) to yield isolated colonies. Bacterial growth was examined after overnight incubation at 37 °C for lactose-fermenting colonies with a morphological appearance typical of *E. coli*. Three such colonies were selected at random (Lidin-Janson et al., 1978), identified by the API 20E system (BioMérieux) and tested for antimicrobial susceptibility by standard agar disk diffusion method (NCCLS, 2000). Selected isolates identified as being other than *E. coli* were excluded from the study, and additional colonies were randomly selected and processed to

eventually obtain three *E. coli* isolates from each sample.

Data from the DPM collected before confirmatory analysis were indicated as DPM raw data and were also subjected to a comparative analysis to investigate their significance if used for rapid screening purposes.

Escherichia coli ATCC 25922 was always used as a reference strain for quality control of susceptibility testing. Results of conventional disk diffusion testing were interpreted as recommended by the NCCLS (2004).

2.3. Statistical analysis

Data were stored and analysed using Epi Info, version 6.04b (CDC, Atlanta, GA, USA). Differences between proportions were statistically assessed using the χ^2 test.

For performance analysis of DPM raw data, sensitivity was defined as the probability of the method to detect antimicrobial resistance of faecal *E. coli* if the resistance trait was truly present, and was calculated by the formula $a/(a+c)$, where a is the number of true positives (i.e. those detected by the conventional method and/or by the DPM after confirmatory analysis) and c is the number of false negatives (i.e. those detected by the conventional method but not by the DPM). Specificity was defined as the probability of the method to test negative for the presence of antimicrobial-resistant faecal *E. coli* if the resistance trait was undetectable, and was calculated by the formula $d/(b+d)$, where d is the number of true negatives (i.e. those in which resistance was not detected by either the conventional method or the DPM) and b is the number of false positives.

3. Results

3.1. Analysis of faecal carriage of antimicrobial-resistant *E. coli* by the DPM with confirmatory analysis and by the conventional method

Faecal samples from 71 subjects were investigated for the presence of resistant *E. coli* using the DPM and the conventional method, as described in the Methods section.

In the DPM after confirmatory analysis, the prevalence of antimicrobial resistance was variable for the different drugs. No resistant isolates were detected for cefotaxime, amikacin and tobramycin, whilst resistant isolates were obtained for the other drugs (Table 1). The highest rates were observed for ampicillin, tetracycline, sulphamethoxazole/trimethoprim and chloramphenicol. Using the conventional method, the overall prevalence of faecal carriage of antimicrobial-resistant *E. coli* was lower than that detected by the DPM, and no isolates resistant to gentamicin and ciprofloxacin were found using the conventional method (Table 1).

Table 1 Prevalence of antimicrobial resistance in commensal *Escherichia coli* isolates from 71 healthy subjects analysed by the direct plating method (DPM) with confirmatory analysis and by the conventional testing method

Antimicrobial	No. of positives (%)	
	DPM with confirmatory analysis	Conventional testing[a]
AMP	57 (80)[b]	37 (52)
CTX	0	0
SXT	47 (66)[c]	34 (48)
F/M	4 (6)	2 (3)
NA	12 (17)[c]	3 (4)
CIP	5 (7)[c]	0
TE	53 (75)[b]	37 (52)
C	27 (38)[c]	16 (23)
AN	0	0
GM	2 (3)	0
NN	0	0

AMP: ampicillin; CTX: cefotaxime; SXT: sulphamethoxazole/trimethoprim; F/M: nitrofurantoin; NA: nalidixic acid; CIP: ciprofloxacin; TE: tetracycline; C: chloramphenicol; AN: amikacin; GM: gentamicin; NN: tobramycin.

[a] Resistance detected in at least one of the three studied colonies.

[b] P-value < 0.01.

[c] P-value < 0.05.

3.2. Definition of interpretive criteria for raw data from the DPM

Using the DPM, either an inhibitory zone (in some cases containing internal colonies) or no inhibitory zone was observed around the antimicrobial-containing disks (Figure 1). Confirmatory testing showed that antimicrobial-resistant *E. coli* isolates were actually present in the majority of cases when the inhibitory zone around the antimicrobial containing disk was absent, or when an inhibitory zone was present but containing internal colonies of coliform morphology (Table 2). Resistance was not confirmed or the isolates were identified as being other than *E. coli* only in a very few cases (5%) of absence of an inhibitory zone or of presence of an inhibitory zone containing internal colonies. On the other hand, when an inhibitory zone without internal colonies was present, *E. coli* isolates

Table 2 Summary of correlations of raw data from the direct plating method with results of confirmatory analysis

Antimicrobial	No inhibition zone		Inhibition zone with internal colonies of coliform morphology		Inhibition zone without internal colonies of coliform morphology	
	No.	Confirmed resistant *E. coli*[a]	No.	Confirmed resistant *E. coli*[b]	No.	Confirmed resistant *E. coli*[c]
AMP	26	25	33	32	12	0
CTX	0	—	0	—	71	0
SXT	22	22	26	26	23	0
F/M	0	—	6	4	66	0
NA	0	—	11	10	60	2
CIP	0	—	6	4	65	1
TE	21	19	34	34	16	0
C	1	1	24	24	46	2
AN	0	—	0	—	71	—
GM	0	—	3	2	68	0
NN	0	—	1	0	70	0

AMP: ampicillin; CTX: cefotaxime; SXT: sulphamethoxazole/trimethoprim; F/M: nitrofurantoin; NA: nalidixic acid; CIP: ciprofloxacin; TE: tetracycline; C: chloramphenicol; AN: amikacin; GM: gentamicin; NN: tobramycin.

[a] Number of cases in which *E. coli* isolates resistant to that antimicrobial agent were detected in the bacterial growth collected close to the disk edge in confirmatory analysis.

[b] Number of cases in which *E. coli* isolates resistant to that antimicrobial agent were detected in the colonies of coliform morphology growing inside the inhibition zone in confirmatory analysis.

[c] Number of cases in which *E. coli* isolates resistant to that antimicrobial were detected in the bacterial growth collected at the edge of the inhibition zone in confirmatory analysis.

Table 3 Distribution of inhibitory zone diameters observed around antimicrobial-containing disks, definition of tentative breakpoints, and performance relative to the direct plating method (DPM) considering raw data

Antimicrobial	Inhibition zone size (mm)[a]				Tentative breakpoint (mm)[b]	TP[c]	FP[d]	TN[e]	FN[f]	Sensitivity DPM raw data (%)[g]	Specificity DPM raw data (%)
	No.	Range	Mean ± SD	Median							
AMP	32	16–28	20 ± 2.6	20	≤13	57	2	10	2	97	83
CTX	56	27–38	33 ± 2.5	32	—	0	—	71	—	—	100
SXT	32	20–31	26 ± 3.8	26	≤10	47	1	18	5	90	95
F/M	58	17–30	25 ± 2.9	25	≤14	4	2	64	1	80	97
NA	58	8–32	24 ± 3.9	24.5	≤13	12	1	58	—	100	98
CIP	56	13–35	29 ± 3.4	30	≤15	5	2	64	—	100	97
TE	36	17–28	21 ± 2.1	21	≤14	53	—	13	5	91	100
C	55	12–35	25 ± 5.4	26	≤12	27	1	37	6	82	97
AN	57	15–22	18 ± 1.7	18	—	0	—	71	—	—	100
GM	59	13–19	16 ± 1.2	15	≤12	2	1	68	—	100	99
NN	57	13–22	15 ± 1.6	14	—	0	—	71	—	—	100

AMP: ampicillin; CTX: cefotaxime; SXT: sulphamethoxazole/trimethoprim; F/M: nitrofurantoin; NA: nalidixic acid; CIP: ciprofloxacin; TE: tetracycline; C: chloramphenicol; AN: amikacin; GM: gentamicin; NN: tobramycin.

[a] Data were calculated on the number of samples reported in the column, regardless of the presence of internal colonies inside the inhibition zone.

[b] Established considering the distribution of inhibition zone sizes, and correlation with results of confirmatory analysis (see Table 2). No tentative breakpoint was proposed when no resistant isolates were detected.

[c] TP: true positives; cases in which resistant *E. coli* were detected by the DPM considering raw data and whose presence was confirmed by confirmatory analysis and/or by the conventional method.

[d] FP: false positives; cases in which resistant *E. coli* were detected by the DPM considering raw data, but whose presence was not confirmed by either confirmatory analysis or the conventional method.

[e] TN: true negatives; cases in which resistant *E. coli* were not detected by the DPM considering raw data and whose absence was confirmed by confirmatory analysis and by the conventional method.

[f] FN: false negatives; cases in which resistant *E. coli* were not detected by the DPM considering raw data, but whose presence was detected by confirmatory analysis and/or by the conventional method.

[g] Sensitivity was calculated only when resistant isolates for an antimicrobial agent were detected.

collected from the edge of the inhibitory zone were usually susceptible to the antimicrobial agent, except for a few cases (nalidixic acid, ciprofloxacin and chloramphenicol) in which the diameter was consistently much smaller than the average size (Table 3). With all agents, measurement of the inhibitory zone diameters (regardless of the presence of internal colonies) revealed a clear monomodal distribution (data not shown). In consideration of this distribution, and of the correlation observed with results of confirmatory analysis, tentative breakpoints could be defined for inhibitory zone diameters (Table 3).

Based on these findings, we elaborated the following interpretive criteria for raw data from the DPM (i.e. those from the plate appearance): (i) the presence of an inhibitory zone around an antimicrobial disk whose diameter is larger than the breakpoint and that does not contain internal colonies of coliform morphology indicates the absence of faecal *E. coli* resistant to that antimicrobial agent; and (ii) the absence of an inhibitory zone around an antimicrobial disk, or the presence of an inhibitory zone whose diameter is smaller than the breakpoint and/or contains internal colonies of coliform morphology indicates the presence of faecal *E. coli* resistant to that antimicrobial agent. Using these simple interpretive criteria, the raw data obtained from the DPM exhibited a sensitivity and specificity ranging, respectively, from 80—100% and from 83—100% (depending on the antimicrobial agent) for the detection of antimicrobial-resistant faecal *E. coli* (Table 3).

3.3. Cost issues

Calculating the costs of the materials needed for performing each method (based on the average costs in the European Union), each sample processed by the conventional method or by the DPM with confirmatory analysis would have cost approximately 28 Euros and 92 Euros, respectively. On the other hand, each sample processed by the DPM to obtain only raw data would have cost approximately 3 Euros. The average working time necessary to process one sample and to obtain results was calculated as 1.30 h, 3 h and 20 min, respectively, for the conventional method, the DPM with confirmatory analysis and the DPM to obtain raw data.

4. Discussion

Information from surveillance of antimicrobial resistance along with data on the use of antimicrobials is considered a powerful tool for the containment of resistance (WHO, 2001). The implementation of surveillance programmes is subordinate to the organisation of healthcare services and the resources available. Reliable data on the magnitude of and trends in antimicrobial resistance are difficult to obtain in low-resource countries where standardised conventional techniques for susceptibility testing are performed only in a very limited number of laboratories.

The development of reliable and low-cost alternative methods could facilitate the implementation of large-scale surveillance in resource-scarce settings.

In this study, a DPM, previously and successfully used in various epidemiological settings to screen resistance in faecal *E. coli* but never standardised, was validated by confirmatory analysis and compared with a conventional method.

As previously reported (Lester et al., 1990; Linton et al., 1972), the DPM showed a higher sensitivity in detecting carriage of resistant *E. coli*. In addition to confirming the higher sensitivity, analysis of the results allowed us to define interpretative criteria for DPM raw data, i.e. those obtained by direct reading of the plate without confirmatory studies. Applying these criteria, the DPM showed good sensitivity and specificity at very low cost.

The DPM, based on the direct application of antimicrobial disks onto the seeded plate, offers many advantages over the alternative method represented by screening techniques using antimicrobial-containing media, both in terms of saving time and materials (plates, media, etc.) and of easier procedures. In our study, confirmatory testing methods included the use of a high quality but expensive identification system for the identification of *E. coli*, such as the API identification system. Cheaper methods based on a short set of biochemical tests (e.g. Kligler Iron Agar, Motility Indole Urea Medium) such as those adopted by many laboratories in low-resource countries would further reduce the costs of the DPM plus confirmatory analysis.

In conclusion, the DPM evaluated in this study proved to be a very sensitive, fast and cheap procedure to investigate the faecal carriage of drug-resistant *E. coli*. Although not specifically tested in this study, the DPM could also be applicable to detect resistance of commensal microbiota of the gut other than *E. coli*. Although the procedure used to perform the DPM is far from the standards for antimicrobial susceptibility testing and is not suitable for clinical use, by virtue of its relative simplicity (application of antimicrobial-containing disks on a MacConkey plate inoculated with a faecal swab),

low cost (one-third of the conventional disk susceptibility method) and rapidity (approximately 14 h), as well as of the remarkable performances, the DPM may represent a useful tool to conduct large-scale resistance surveillance studies and to monitor resistance control programmes in a cost-effective manner. It seems especially attractive for use in low-resource countries.

Conflicts of interest statement
The authors have no conflicts of interest concerning the work reported in this paper.

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References

Bartoloni, A., Cutts, F., Leoni, S., Austin, C.C., Mantella, A., Guglielmetti, P., Roselli, M., Salazar, E., Paradisi, F., 1998. Patterns of antimicrobial use and antimicrobial resistance among healthy children in Bolivia. Trop. Med. Int. Health 3, 116—123.

Bartoloni, A., Bartalesi, F., Mantella, A., Dell'Amico, E., Roselli, M., Strohmeyer, M., Gamboa Barahona, H., Prieto Barrón, V., Paradisi, F., Rossolini, G.M., 2004. High prevalence of acquired antimicrobial resistance unrelated to heavy antimicrobial consumption. J. Infect. Dis. 189, 1291—1294.

Calva, J.J., Sifuentes-Osornio, J., Cerón, C., 1996. Antimicrobial resistance in fecal flora: longitudinal community-based surveillance of children from urban Mexico. Antimicrob. Agents Chemother. 40, 1699—1702.

Datta, N., 1969. Drug resistance and R factor in the bowel bacteria of London patients before and after admission to hospital. Br. Med. J. 2, 404—411.

Guzmán-Blanco, M., Casellas, J.M., Sader, H.S., 2000. Bacterial resistance to antimicrobial agents in Latin America. Infect. Dis. Clin. North Am. 14, 67—81.

Hart, C.A., Kariuki, S., 1998. Antimicrobial resistance in developing countries. Br. Med. J. 317, 647—650.

Kariuki, S., Hart, C.A., 2001. Global aspects of antimicrobial-resistant enteric bacteria. Curr. Opin. Infect. Dis. 14, 579—586.

Lester, S.C., Del Pilar Pla, M., Wang, F., Perez Schael, I., Jiang, H., O'Brien, T.F., 1990. The carriage of *Escherichia coli* resistant to antibiotic agents by healthy children in Boston, in Caracas, Venezuela and in Qin Pu, China. N. Engl. J. Med. 323, 285—289.

Levin, B.R., Lipsitch, M., Perrot, V., Schrag, S., Antia, R., Simonsen, L., Walker, N.M., Stewart, F.M., 1997. The population genetics of antibiotic resistance. Clin. Infect. Dis. 24 (Suppl. 1), S9—S16.

Lidin-Janson, G., Kaijser, B., Lincoln, K., Olling, S., Wedel, H., 1978. The homogeneity of the faecal coliform flora of normal school-girls, characterized by serological and biochemical properties. Med. Microbiol. Immunol. (Berl.) 164, 247—253.

Linton, K.B., Lee, P.A., Richmond, M.H., Gillespie, W.A., 1972. Antibiotic resistance and transmissible R-factors in the intestinal coliform flora of healthy adults and children in an urban and a rural community. J. Hyg. (Lond.) 70, 99—104.

Moorhouse, E.C., 1969. Transferable drug resistance in enterobacteria isolated from urban infants. Br. Med. J. 2, 405—407.

NCCLS, 2000. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, PA.

NCCLS, 2004. Performance Standards for Antimicrobial Susceptibility Testing; 14th Informational Supplement. National Committee for Clinical Laboratory Standards, Wayne, PA, NCCLS document M100-S14.

Okeke, I.N., Fayinka, S.T., Lamikanra, A., 2000. Antibiotic resistance in *Escherichia coli* from Nigerian students, 1986—1998. Emerg. Infect. Dis. 6, 393—396.

Österblad, M., Hakanen, A., Manninen, R., Leistevuo, T., Peltonen, R., Meurman, O., Huovinen, P., Kotilainen, P., 2000. A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. Antimicrob. Agents Chemother. 44, 1479—1484.

Rodríguez, A.J., Niño Cotrina, R.A., Neyra Pérez, C., Rodríguez, C.N., Barbella, R., Lakatos, M., Molina, N., García, A., Duque, C., Meijomil, P., 2001. Comparative study of antimicrobial resistance of *Escherichia coli* strains isolated from urinary tract infection in patients from Caracas and Lima. J. Antimicrob. Chemother. 47, 903—904.

Shanahan, P.M.A., Wylie, B.A., Adrian, P.V., Koornhof, H.J., Thomson, C.J., Amyes, G.B., 1993. The prevalence of antimicrobial resistance in human faecal flora in South Africa. Epidemiol. Infect. 111, 221—228.

Shears, P., 2001. Antibiotic resistance in the tropics. Trans. R. Soc. Trop. Med. Hyg. 95, 127—130.

WHO, 2001. Global Strategy for Containment of Antimicrobial Resistance. http://www.who.int/emc-documents/antimicrobial_resistance/docs/EGlobal_Strat.pdf [accessed January 2005].