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ARTICLE

Changing microbiological profile of pathogenic bacteria in diabetic foot infections: time for a rethink on which empirical therapy to choose?

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Abstract
Aims/hypothesis We studied the bacterial aetiology and antibiotic sensitivity pattern of diabetic foot ulcers in India.
Methods Records of 447 hospitalised patients between 1991 and 2008 were retrospectively analysed between two time periods (before and after 1999) to compare bacterial aetiology and antimicrobial sensitivity patterns. The first three consecutive cultures from the same wound during treatment were evaluated.
Results Of 1,632 cultures, 66% were polymicrobial, 23% monomicrobial and 11% sterile. In the monomicrobial group, 14% (n=228) of cultures were Gram-negative, whereas 9% (n=147) were Gram-positive. The most common pathogens in the first culture were *Pseudomonas aeruginosa* (20.1%), *Staphylococcus aureus* (17.2%) and *Escherichia coli* (16.3%). Results for the third cultures showed persistence of *P. aeruginosa* (15.3%) and *E. coli* (14.2%). Gram-negative isolates dominated over Gram-positive ones (25.3% vs 15.1%, p<0.05). Antibiotic sensitivity patterns before and after 1999 were: piperacillin–tazobactam 74% vs 66% (p<0.005), imipenem 77% vs 85% (NS), cefoperazone–sulbactam 47% vs 44% (p<0.005), amikacin 62% vs 78% (NS), ceftriaxone 41% vs 36% (p<0.005), amoxicillin–clavulanate 51% vs 43% (p<0.05) and clindamycin 43% vs 36% (p<0.005), respectively.
Conclusions/interpretation Unlike in the West, in India Gram-negative bacteria were found to have always been dominant in the wounds of patients with diabetic foot infections. Infection with polymicrobial multidrug-resistant Gram-negative bacilli is common. The policy of empirical antimicrobial therapy at tertiary care needs to be changed.

Keywords Bacterial aetiology · Diabetic foot infections · Empirical therapy · Multidrug-resistant organism

Abbreviations

DFI	Diabetic foot infection
ESBL	Extended spectrum β-lactamase
MDRO	Multidrug-resistant organism
MRSA	Methicillin-resistant *S. aureus*

Introduction

Foot infections in patients with diabetes are initially treated empirically and involve vital decisions regarding severity of infection, route of administration, co-morbidities and spectrum of organisms to be covered [1–4]. Therapy directed at known causative organisms can significantly improve the outcome and reduce infection-related morbidities. Reports from western countries have found that *Staphylococcus aureus* and β-haemolytic streptococci are the main causative pathogens [4–11]. In India, the choice of empirical antimicrobials is extrapolated from data available from western countries, which may or may not be appropriate for Indian patients [7–9, 12]. Since long-term studies from India on antimicrobial resistance and bacterial aetiology of diabetic foot infections (DFI) are scarce, we

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analysed existing hospital data on the microbiological profile of pathogenic bacteria isolated from wounds of patients with DFI over a period of time. Our study aims to identify the pathogenic bacteria and the changes in their antimicrobial sensitivity pattern in two time periods (up to year 1999 and thereafter).

Methods

Background From 1991 to 2008, a total of 447 DFI patients were hospitalised in the Endocrine Surgery ward of Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, a tertiary care centre. Approval for this study was given by the Ethics Committee of the institute. Inclusion criteria included patients with DFI who were hospitalised for surgical management of foot ulcer. The complete data from 434 patients were available for inclusion in this retrospective study. All patients with incomplete data or who were not admitted for surgical intervention for treatment of foot ulcer were excluded.

Diabetic foot ulcers were graded using Wagner's classification prior to 1999 and by the University of Texas classification after 1999 [13, 14]. A questionnaire was designed, which included patient variables such as age, sex, duration of diabetes, glycaemic control, presence or absence of retinopathy, nephropathy (serum creatinine or presence of micro- or macro-albuminuria), neuropathy, peripheral vascular disease, cardiac abnormalities, gastropathy and duration of hospital stay.

A record was made of foot ulcer grade, duration of ulcer, infecting bacteria, and their culture and sensitivity profile including the antimicrobial agents used and duration of treatment. Data were then divided into two groups to observe changes in microbiological profile and sensitivity patterns over time. Group 1 included patients admitted between years 1991 and 1999; group 2 included patients admitted between 2000 and 2008.

Method for procurement of culture specimens After admission to the ward, a minimum of three specimens were obtained from the wound for culture as follows: (1) at the time of admission; (2) at the time of first debridement; and (3) on subsequent debridement, i.e. weekly or as and when done. Specimens (pus, wound exudates or tissue biopsy) for microbiological studies were obtained from the ulcer region. To avoid isolation of colonising flora, the wound was first thoroughly cleaned with normal saline, after which samples were obtained from deeper pockets. Pus and exudates were collected from the margins and base of the ulcer, either in a syringe or using a sterile swab stick, which was then transported in a clean and sterile test-tube. Tissue biopsy/debrided fragments or a wedge of tissue was obtained during debridement using a sterile blade/knife from the base and/or margin of the ulcer, and transported in a sterile solution of normal saline. All specimens were transported without delay to the hospital's Microbiology Laboratory for further processing.

Culture and antibiotic sensitivity testing For aerobic culture, specimens were inoculated onto 5% (vol./vol.) sheep blood agar and MacConkey agar and incubated at 37°C for 48 h. For anaerobic culture, specimens were inoculated on to Wilkin's Chalgreen agar (laced with kanamycin and vancomycin) and plates were incubated for 5 days in an anaerobic chamber at 37°C.

Disc diffusion testing was carried out for all the isolates as currently recommended by the Clinical and Laboratory Standards Institute [15]. Gram-negative bacilli were tested for extended spectrum β-lactamase (ESBL) production and *Staphylococcus* species were tested for methicillin resistance by using 1 μg oxacillin disc. Multidrug-resistant organisms (MOROs) were defined as methicillin-resistant *S. aureus* (MRSA), bacteria producing ESBL and methicillin-resistant coagulase-negative staphylococci. Appropriate quality controls were used where indicated.

Statistical analysis Data were analysed using SPSS software (version 16; SPSS, Chicago, IL, USA) and test of proportion was applied to calculate any significant differences ($p<0.05$ considered statistically significant).

Results

Data from 434 hospitalised patients with DFI were analysed. The demographic details and comorbid conditions are shown in Table 1. The details of ulcers with

Table 1 Clinical details of 434 patients infected with diabetic foot ulcers

Characteristic	Value
Mean age (years)	56±9.84
Mean foot ulcer duration (days)	16±7.4
Men/women ratio	4:1
Retinopathy, *n* (%)	334 (77)
Cardiopathy, *n* (%)	312 (72)
Nephropathy, *n* (%)	282 (65)
Neuropathy, *n* (%)	252 (58)
Gastropathy, *n* (%)	117 (27)
Vasculopathy, *n* (%)	313 (72)
Poor glycaemic control, *n* (%) [a]	249 (57)

Unless otherwise stated, values are mean ± SD

[a] $HbA_{1c} \geq 8.0\%$

classification systems (Wagner's prior to 1999, University of Texas thereafter) are shown in Table 2. All patients underwent surgery in the form of debridement or minor/major amputation. Other procedures included revision debridement and skin grafting.

The first three consecutive culture specimens from 434 patients yielded a total of 1,632 cultures, which were then analysed. The mean time interval (±SEM) between first two cultures was 3.67 days (±0.04); between the second and third cultures the interval was 5.61 days (±0.06). Out of 1,632 cultures, 66% (n=1,093) were polymicrobial, whereas 23% were monomicrobial (n=375). In the monomicrobial group, 14% (n=228) of cultures were Gram-negative; 9% (n=147) were Gram-positive. Cultures were sterile in 10.8% (n=179) specimens. First culture results proved that Gram-negative organisms dominated over Gram-positive ones (25.3% vs 15.1%, $p<0.05$). The majority of the organisms were aerobes, but anaerobes were also isolated. Patient samples were sub-classified into two groups based on time periods of study (up to the year 1999 and thereafter). Table 3 describes the frequency of isolates obtained from cultures in both the above groups over two time periods (before and after 1999). Table 3 also shows that *P. aeruginosa* and *E. coli* persisted in all three cultures taken over a period of time during the entire treatment course, indicating a resistance pattern. Figure 1 (Gram-negative organisms) and Fig. 2 (Gram-positive organisms) indicate that resistance to antibiotics increased in isolates recovered from three consecutive cultures. Tests of significance when applied to these data are shown in separate Tables 4 and 5.

Commonly used empirical antimicrobial agents or their combinations included cloxacillin, metronidazole and amoxicillin–clavulanate in the first study period, whereas amoxicillin–clavulanate, clindamycin, pipearacillin–tazobactam or cefoperazone–sulbactam drugs or combinations were most commonly used in the second period. However, these drugs were suitably modified as indicated by subsequent culture and sensitivity reports.

The resistance profile of isolates obtained from first cultures in both time periods is shown in Fig. 3, which depicts a rising trend in the resistance pattern to commonly used antimicrobial agents in the long term. Multidrug-resistant organisms in this study constituted up to 81%, with 56% of *E. coli* being ESBLs and 25% of *S. aureus* being methicillin-resistant (MRSA).

Table 2 Number of patients (%) in the Wagner and UT classification systems

Classification system	Patients, *n* (%)
Wagner classification grade (before 1999)	164
Grade 1	22 (13.4)
Grade 2	46 (28.0)
Grade 3	41 (25)
Grade 4	35 (21.3)
Grade 5	20 (12.2)
University of Texas classification (after 1999)	270
Stage A	
Grade 1	8 (2.9)
Grade 2	20 (7.4)
Grade 3	22 (8.1)
Stage B	
Grade 1	12 (4.4)
Grade 2	26 (9.6)
Grade 3	24 (8.8)
Stage C	
Grade 1	14 (5.2)
Grade 2	20 (7.4)
Grade 3	28 (10.4)
Stage D	
Grade 1	18 (6.6)
Grade 2	30 (11.1)
Grade 3	48 (17.7)

Discussion

Severe and moderate DFIs are usually polymicrobial in nature, whereas mild DFI are mostly monomicrobial [2, 4, 9–11]. In our study, 20.2% of the patients had mild infection. First culture results were monomicrobial in 78% of cases with mild DFI, while they were polymicrobial in 73% of cases with moderate to severe infections. Studies from western countries show that Gram-positive aerobes are the predominant organisms isolated from DFI [4–11]. In contrast, two recent Indian studies have shown a preponderance of Gram-negative aerobes. Gadepalli et al., in their study on 80 ulcer specimens, recovered 183 isolates, of which 28.7% were Gram-negative and only 13.8% Gram-positive [16]. Shankar et al. also reported Gram-negative aerobes to be the most frequently isolated pathogens (51.4%), followed by Gram-positive aerobes (33.3%) and anaerobes [17]. Studies from Malaysia have also reported a predominance of Gram-negative bacteria (52%) in patients with DFI, the most common pathogens isolated being *Proteus* spp., *Klebsiella pneumoniae*, *E. coli* and *Enterobacter cloacae* [18]. The difference observed in the prevalence of Gram-negative bacilli in DFI between diabetic patients from eastern and western countries remains largely unknown. However, environmental factors such as sanitary habits, e.g. use of water for peri-anal wash (ablution) after defaecation leading to contamination of hands with faecal flora, are proposed to be responsible for increased Gram-

Table 3 Frequency of isolates in various cultures up to year 1999 and thereafter

Variable	Isolates per culture				Overall isolates	
	1st culture	2nd culture	3rd culture	All cultures	Up to 1999	After 1999
Cultures (*n*)	691	580	361	1632	689	943
Gram-negative	415 (25.3)	332 (20.3)	186 (11)	932 (57.1)	349 (50.6)	603 (64)
P. aeruginosa	135 (20.1)	89 (15.3)	53 (15.3)	277 (16.9)	123	147
E. coli	116 (16.3)	97 (16.7)	50 (14.2)	263 (16.1)	70	153
Proteus spp.	63 (3.8)	54 (3.3)	27 (1.7)	143 (8.8)	62	61
Klebsiella spp.	48 (2.9)	40 (2.5)	21 (1.3)	109 (6.7)	20	89
Citrobacter spp.	21 (1.3)	21 (1.3)	10 (0.6)	52 (3.2)	10	42
Acinetobacter spp.	19 (1.2)	23 (1.4)	18 (1.1)	60 (3.7)	12	48
Enterobacter spp.	13 (0.8)	8 (0.5)	7 (0.4)	28 (1.7)	52	63
Gram-positive	247 (15.1)	182 (11.2)	82 (5.0)	511 (31.3)	280 (40.6)	286 (30.3)
S. aureus	116 (17.2)	79 (13.6)	30 (1.8)	225 (13.8)	101	144
Enterococcus spp.	77 (4.7)	52 (3.2)	26 (1.6)	155 (9.5)	92	83
Coagulase-negative *Staphylococcus*	30 (1.8)	33 (2.0)	19 (1.2)	82 (5.0)	45	37
Streptococcus spp.	24 (1.5)	18 (1.1)	7 (0.4)	49 (3.0)	27	22
Anaerobes	7 (0.4)	3 (0.2)	2 (0.1)	12 (0.7)	5	7
Sterile	22 (1.3)	63 (3.9)	91 (5.6)	177 (10.8)	55	47

Unless otherwise stated, values are mean. Values in parentheses are per cent (%)

negative infections in the developing world compared with the West. Similar findings have been reported in continuous ambulatory peritoneal dialysis (CAPD) peritonitis by Prasad et al. [19].

Data from our study (Table 1) show that Gram-negative organisms were the major infective pathogens as compared with Gram-positive isolates (51.7% vs 31.3%; $p<0.01$) in both time periods. Of all the aerobic bacteria recovered, the percentage of Gram-negative isolates increased from 50.6% before 1999 to 64% after 1999 ($p<0.01$).The most common isolate in our study was *P. aeruginosa* (16.9%) followed by *E. coli* (16.1%) and *Proteus* spp. (8.8%). Other Gram-negative aerobes recovered were *Citrobacter* spp., *Enterobacter* spp. and *Acinetobacter* spp.

The significance of separate analysis of cultures 1, 2 and 3 in the same patient is that it enables the documentation of

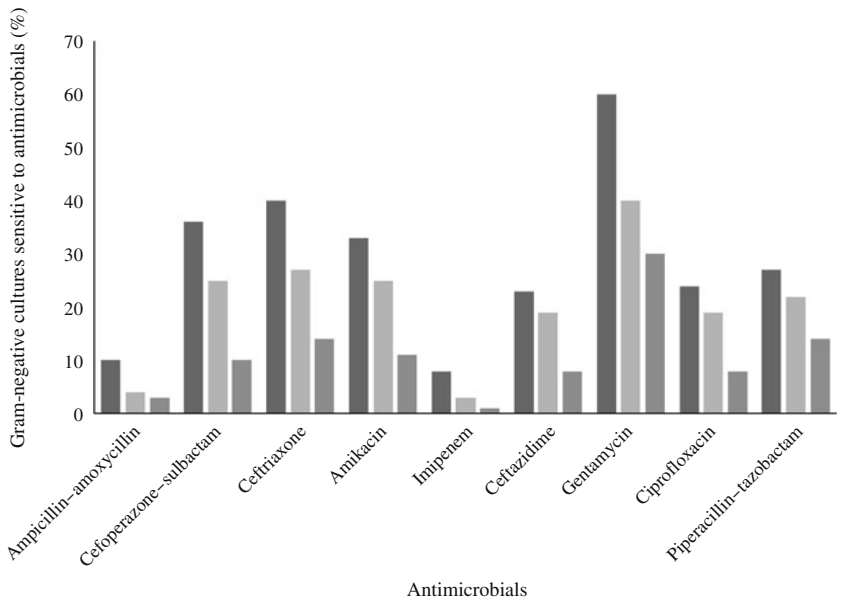

Fig. 1 Overall antimicrobial sensitivity profile of Gram-negative bacterial cultures. Dark grey, culture 1; light grey, culture 2; medium grey, culture 3

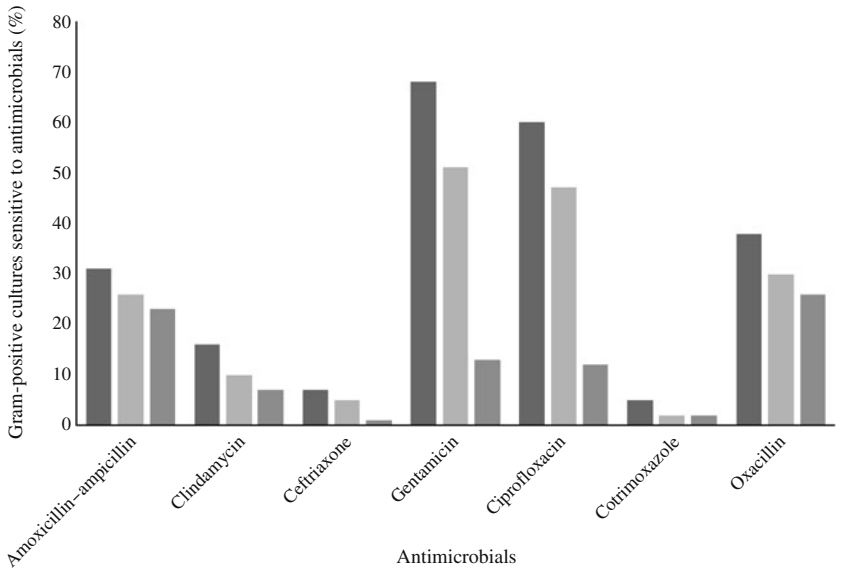

Fig. 2 Antimicrobial sensitivity profile of Gram-positive bacteria. Dark grey, culture 1; light grey, culture 2; medium grey, culture 3

the emergence of resistance among Gram-negative and Gram-positive bacteria against commonly used antimicrobials (Figs 1 and 2, Tables 4 and 5). Follow-up cultures in the same patient at the time of admission, at debridement and at discharge also allowed us to monitor the change of microbial flora during treatment. Culture and sensitivity profile of isolates from second and third cultures showed persistence of *P. aeruginosa* and *E. coli* in up to 20% of isolates, which is worrisome as this indicates increasing resistance despite aggressive medical therapy. Shankar et al. also reported that *P. aeruginosa* was the most common bacterial isolate in their study [17]. Bansal et al., in their study on microbial flora in diabetic foot ulcers, reported that 76% of the isolates were Gram-negative, with *P. aeruginosa* being the most common pathogen [20].

Figure 3 shows a comparison of pre- and post-1999 populations that indicates increasing resistance to the more commonly used antimicrobial agents.

Recent studies from India have reported the prevalence of ESBL producers to be between 55% and 70% [21, 22]. MDROs in our study accounted for around 81%, with 56% of *E. coli* isolates being ESBL producers. This is consistent with the report of Gadepalli et al. [16]. The persistence of *P. aeruginosa* and *E. coli* in third culture isolates confirms that multi-drug-resistant pathogens are extremely common in hospitalised patients with DFI, despite appropriate choice of antimicrobials. The high rates of antibiotic resistance observed in the present study may be due to the fact that ours is a tertiary care hospital as we see patients after the referring hospital has already tried and failed to control infection using a combination of different antimicrobials. Since facilities for microbiological studies at the first contact physician/surgeon are usually not available in district hospitals/smaller cities in India, indiscriminate antimicrobial therapy (i.e. without establishing the aetiology of the infection) eradicates susceptible Gram-positive cocci and, as a result, selects resistant

Table 4 Gram-negative bacteria: statistical analysis of sensitivity pattern

Antimicrobial agent	*p* values per cultures		
	Culture 1 vs culture 2	Culture 2 vs culture 3	Culture 1 vs culture 3
Ampicillin/amoxicillin	0.001*	0.56	0.001*
Cefoperazone/sulbactam	0.59	0.006 *	0.003*
Ceftriaxone	0.02*	0.98	0.09
Amikacin	0.03 *	0.09	0.03*
Imipenem	0.1	0.34	0.59
Ceftazidime	0.03*	0.09	0.03*
Gentamicin	0.02*	0.98	0.09
Ciprofloxacin	0.09	0.01*	0.01*
Piperacillin–tazobactam	0.42	0.2	0.03*

*Statistically significant ($p \leq 0.05$)

Table 5 Gram-positive bacteria: statistical analysis of sensitivity pattern

Antimicrobial agent used	*p* values per cultures		
	Culture 1 vs culture 2	Culture 2 vs culture 3	Culture 1 vs culture 3
Amoxicillin–clavulanate	0.18	0.24	0.8
Clindamycin	0.53	0.53	0.67
Ceftriaxone	0.36	0.04*	0.36
Gentamicin	0.19	0.01*	0.01*
Ciprofloxacin	0.35	0.64	0.21
Cotrimoxazole	0.16	0.61	0.64
Oxacillin	0.26	0.40	0.07

*Statistically significant ($p \leq 0.05$)

Gram-positive cocci and Gram-negative bacilli, as well [23, 24]. The recent emergence of other mechanisms of drug resistance in Gram-negative bacilli such as metallo-β-lactamase (MBL) production and Amp C enzymes also needs to be explored [25]. All this is disconcerting because infection with these organisms limits the choice of antibiotic treatment and may lead to a worse outcome. These findings are important for patient management and underscore the need for institutional infection control committees to develop antibiotic treatment policies.

Several studies have also investigated the relationship between the specimen collection method and both numbers and types of organisms recovered from infected wounds. Some have found that tissue specimens are more sensitive and specific, containing fewer apparent contaminants and more pathogens than swab cultures [26, 27]. Others have reported that with adequate preliminary debridement, the use of a wound swab is as reliable as the use of a tissue specimen [27, 28]. In our study, swab specimens were collected only after thorough cleaning with sterile normal saline, after debridement of the wound and before application of an antiseptic agent. Culture material obtained from deeper tissues only was sent for microbiological study. However, sample collection procedures need to be carefully defined and observed, as skin contaminants may confuse the microbial profiles, possibly resulting in misinterpretation of culture reports with adverse effects on clinical decisions. Casual swab cultures should be strictly forbidden.

In conclusion, this study presents a comprehensive temporal microbiological survey of infected diabetic foot ulcers in hospitalised patients. Unlike in the West, in India we found that Gram-negative bacteria dominated in DFI patients. Hence all patients with DFI admitted to a tertiary care hospital in India may not require empirical therapy for Gram-positive coverage. A β-lactam agent with/without inhibitor combination or a quinolone as an empirical agent after establishing the patient's history of previous antibiotic usage would probably be more appropriate. However, treatment modes can be modified based on the severity of infection and on any available microbiological data such as recent culture results or current Gram-stained smear findings. Patients who are positive for MRSA, if the infection is community-acquired and sensitive to clindamycin, can be treated with this drug. In hospital-acquired infections with MRSA, glycopeptides are usually the drug of choice. In the event of *Pseudomonas* infection, an anti-pseudomonal drug can be added. In our study, piperacillin–tazobactam/cefoperazone–sulbactam adequately covered

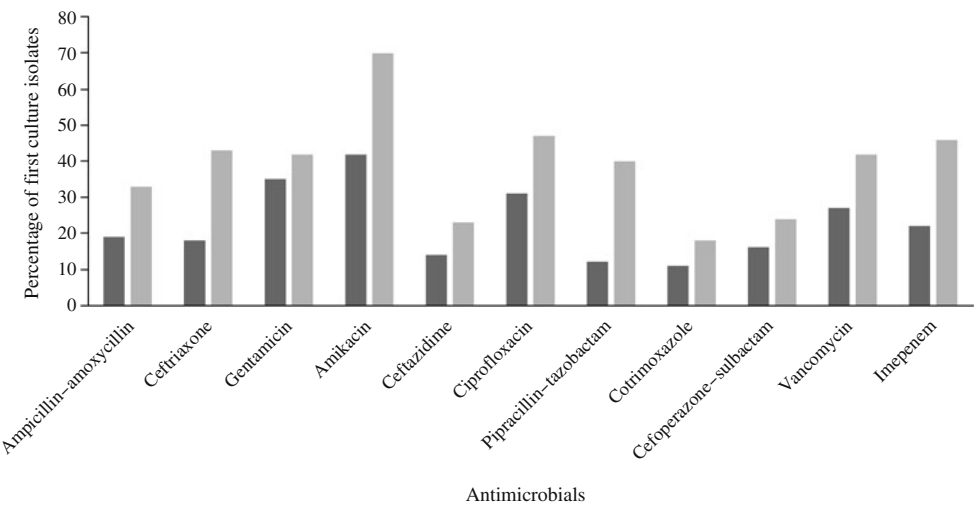

Fig. 3 First culture isolates. Comparison of resistance pattern of pathogens isolated in two time periods, i.e. up to 1999 (dark grey) and after 1999 (light grey)

such infection. The results of this study therefore alert us to the need for proper management of antibiotics to optimise patient care and improve clinical outcome.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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