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Antimicrobial resistance among anaerobes isolated from clinical specimens in Kuwait hospitals: Comparative analysis of 11-year data

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ABSTRACT

Our objective was to compare the antimicrobial resistance trends among clinically relevant anaerobes against 9 different antibiotics over two periods, 2008–2012 and 2002–2007. Antimicrobial susceptibility testing was performed by determining the MICs using *E* test method. The interpretation of results was according to the breakpoints recommended by the Clinical Laboratory and Standard Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST). A total of 2240 clinically significant isolates were collected between 2008 and 2012 in four teaching hospitals in Kuwait. The commonest isolates were *Bacteroides fragilis* (40.4%), *Prevotella bivia* (18.6%), *Peptostreptococcus* spp. (13.8%) and *Bacteroides ovatus* (11.1%). According to CLSI and EUCAST breakpoints used for the 2008–2012 and 2002–2007 isolates, high resistance rates to amoxicillin–clavulanic acid, clindamycin, penicillin and piperacillin were noted among the Gram-negative isolates. They ranged between 0 and 0–62.1 and 62.1%, and 0 and 0–59.1 and 62.1%, respectively against clindamycin, 0 and 0–34.5 and 45.3%, and 0 and 0–45 and 57.5%, respectively against piperacillin and 0 and 0–24.2 and 24.2%, and 0 and 0–23.1 and 30.6%, respectively against amoxicillin–clavulanic acid. The mean interpretative results by both CLSI and EUCAST during the 2008–2012 and 2002–2007 periods showed that the *B. fragilis* isolates were highly resistant to penicillin (100 vs 100%), clindamycin (43.7 vs 44.2%), piperacillin (35.8 vs 42.7%) and amoxicillin–clavulanic acid (13.2 vs 14%), respectively. When compared with 2002–2007, the CLSI, but not EUCAST, demonstrated statistically significant decreased resistance to clindamycin ($P < 0.03$). However, both interpretative criteria showed demonstrable statistically significant decrease in resistance rates to imipenem ($P < 0.00097$ vs $P < 0.00074$), meropenem ($P < 0.000006$ vs $P < 0.0407$) and piperacillin ($P < 0.000017$ vs $P < 0.0461$). Our data shows that there is a need for periodic monitoring of the susceptibility testing for anaerobic bacteria in the face of increasing resistance rates as well as to guide in the empirical therapy of anaerobic infections.

1. Introduction

Anaerobes are important part of bacterial flora of the skin and mucous membranes. They are common cause of various endogenous infections in different parts of the body and are often involved in many polymicrobial infections such as intra-abdominal and other post-operative wound infections, obstetric/gynecological, oro-dental and brain abscesses, as well as skin and soft tissue infections including diabetic foot infections [1,2]. Management of these severe or life-threatening infections with a high probability of the presence of anaerobes is often dependent on empirical therapy. Because anaerobic cultures are cumbersome, costly and technically difficult, susceptibility testing is often not done routinely in Clinical Microbiology Laboratory. Anaerobes are becoming resistant to some of the notable anti-anaerobic drugs. Experiences in several institutes in the world have confirmed that antibiotic resistance among anaerobes has been on ascendancy in the last decade [3–5]. Antibiotic resistance to anaerobes includes those antibiotics that were considered to be active against anaerobes such as carbapenems and metronidazole [4,6]. In addition, clinical failures have been observed in patients who were treated with ineffective anti-anaerobic antimicrobial agents [7,8]. Since several Clinical Microbiology Laboratories are not performing antimicrobial susceptibility testing for anaerobes routinely, it is important to do regular surveillance on clinically significant anaerobes in order to guide the physician who are giving empirical treatment for infections involving anaerobes. The objective of this study was to investigate the trends of antibiotic resistance in clinically relevant isolates of

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anaerobes over a period of 5 years (2008–2012) in Kuwait hospitals and compare it with the results from previous study in Kuwait (2002–2007) [4].

2. Material and methods

2.1. Bacterial strains

Non-repetitive (one isolate per patient) clinically significant isolates submitted to the Anaerobe Reference Laboratory (ARL) over a period of 5 years (2008–2012) were included in the study. The strains were collected from four different tertiary care teaching hospitals in Kuwait (Amiri, Ibn Sina, Maternity and Mubarak hospitals). The specimen sources and patients biodata were carefully recorded for each isolate. Processing of the clinical specimens was performed in the respective microbiology departments according to their local protocols. Then, the isolates were sent to ARL for further identification and susceptibility testing. In the ARL, they were identified by API 20A (bioMerieux, Marcy I'Etoile, France), VITEK MS (MALDI-TOF MS; bioMerieux), and when indicated, PCR and sequencing.

2.2. Antimicrobial susceptibility testing (AST)

Minimum inhibitory concentrations (MICs) of 9 anti-anaerobic antibiotics were determined using the *E* test method (bioMerieux) according to manufacturer's instructions. The antibiotic tested was the following: amoxicillin–clavulanic acid, clindamycin, imipenem, meropenem, metronidazole, penicillin, piperacillin, piperacillin–tazobactam, and vancomycin (for Gram-positive only). Freshly-prepared Brucella agar (Becton Dickinson, Heidelberg, Germany) supplemented with hemin (5 μg/ml), vitamin K_1 (1 μg/ml) and laked sheep blood (5% v/v) was used as recommended by Clinical Laboratory Standards Institute (CLSI) [9]. The inoculated plates were incubated at 37 °C, in anaerobic environment of N_2 80%, H_2 10%, CO_2 10%, for 48 h using the Anoxomat WS800 anaerobic apparatus (MART Microbiology BV, Lichtenvoorde, Netherlands). Resistance profiles of the isolates were determined according to the interpretative criteria recommended by the CLSI, 2014 [9] and EUCAST, 2014 breakpoints [10]. *Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, for Gram-negative organisms, and *Clostridium difficile* ATCC 700057 and *Eubacterium lentum* ATCC 43055 for Gram-positive organisms, were included as controls in each run. Results for the isolates were accepted if the quality control strains results were within the established CLSI ranges [9]. MIC_{50} and MIC_{90} were calculated as MIC that inhibited 50 and 90% of the isolates, respectively. The CLSI and EUCAST [10] breakpoints for the anti-anaerobic antimicrobial agents tested against the clinical isolates are shown in Table 1.

2.3. Ethical approval

No ethical approval was required as this was just routine data collection and analysis of the results.

2.4. Statistical analysis

The EpiCale 2000, version 1.02 (Brixton Health, Llanidloes, Powys, Wales, UK) was used to compare two proportions–percentages with 95% confidence interval and one sided *P*-value.

3. Results

The sources of the clinically significant isolates are shown in Table 2. They were from blood cultures, 113 (5%); wounds 1003 (44.8%); tissue 134 (6%); gynecological and obstetrical sites, 412 (18.4%); lower respiratory tract, 50 (2.2%), and pus and fluid aspirate, 528 (23.6%).

As shown in Table 3, a total of 2240 clinically significant isolates were collected from different sources during 2008–2012, of which 1925 (85.9%) were Gram-negative bacteria and 315 (14.1%) Gram-positive bacteria. The top 6 isolates encountered in 2002–2007 versus 2008–2012 periods were *B. fragilis*, 32.8 vs 40.4%; *Bacteroides ovatus*, 13.8 vs 11.1%; *Prevotella bivia*, 10.8 vs 18.6%; *Peptostreptococcus* spp., 19.6 vs 13.8%; *Prevotella oralis*, 7.6 vs 6.4%; and *Bacteroides* spp., 7.2 vs 4.4%, respectively.

The MIC ranges (MIC_{50}s and MIC_{90}s), percentage of resistant isolates according to CLSI and EUCAST breakpoints and the *P* values of the differences in the resistance rates of isolates between 2002–2007 versus 2008–2012 are represented together in Table 4 in order to allow a valid comparison of the results with previous surveillance data. These were re-calculated from our original published data [4] with the modified breakpoints.

According to the CLSI breakpoints for metronidazole, the majority of the isolates were susceptible with the exception of *B. fragilis* whose resistance rates were 2.7% in 2008–2012 vs 1.5% in 2002–2007 ($P > 0.05$) and *B. ovatus* 5.6% vs 0%, respectively ($P < 0.000013$; CI 95% [2.40, 8.80]). With EUCAST breakpoints, the resistance rates increased significantly to 3 vs 1.5%, respectively for *B. fragilis* ($P < 0.0267$; CI 95% [0.00, 3.00]) but non-significantly for *B. ovatus* 6.0 vs 5.4%, respectively ($P > 0.05$).

Table 1
[b]EUCAST and CLSI breakpoints for the tested antimicrobial agents against anaerobes.

	EUCAST				CLSI		
	Gram-positive		Gram-negative				
	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Intermediate	Resistant
Amox–clav	[a]NA	[a]NA	≤4/2	>8/2	≤4/2	8/4	≥16/8
Clindamycin	[a]NA	[a]NA	≤4	>4	≤2	4	≥8
Imipenem	≤2	>8	≤2	>8	≤4	8	≥16
Meropenem	≤2	>8	≤2	>8	≤4	8	≥16
Metronidazole	≤4	>4	≤4	>2	≤8	16	≥32
Penicillin	[a]NA	[a]NA	≤0.25	>0.5	≤0.5	1	≥2
Piperacillin	[a]NA	[a]NA	≤16	>16	≤32	64	≥128
Pip–taz	[a]NA	[a]NA	≤8	>16	≤32/4	64/4	≥128/4
Vancomycin	≤2	>8	–	–	NA	NA	NA

Amox–clav = amoxicillin–clavulanic acid; Pip–taz = piperacillin–tazobactam.
[a] NA = not available. In the EUCAST Table, the intermediate category is not listed.
[b] EUCAST, Clinical Breakpoints Tables v. 4.0, for interpretation of MICs.