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Susceptibility testing methods

Antimicrobial susceptibility of clinically significant anaerobic bacteria in Slovenia, 2014

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Background: Routine antimicrobial susceptibility testing (AST) of anaerobic bacteria is rarely performed in microbiological laboratory despite the fact that anaerobes are well established pathogens responsible for severe human infections. Recent advances in anaerobic culture processing with simplified identification with MALDI-TOF and AST standardization have narrowed the gap between aerobic and anaerobic bacteriology. The aim of the study was to collect routine AST data and compare them with available results from previous surveillance studies.

Material/methods: Routine AST results for anaerobic bacteria isolated in 2014 from various specimen sources at a central Slovenian microbiological laboratory were analysed. MALDI-TOF was used for species identification. Minimal inhibitory concentration (MIC) for 5 antimicrobial agents with activity against anaerobes (i.e. penicillin, co-amoxiclav, imipenem, clindamycin and metronidazole) was determined with gradient diffusion test using supplemented Brucella agar and recommendations by the CLSI. The EUCAST clinical breakpoints were used for the interpretation of MIC results. Anaerobic atmosphere was created with gas exchange Anoxomat system in jars. *Bacteroides fragilis* ATCC 25285 was used for quality control of AST.

Results: A total of 2.600 non-repetitive clinically important anaerobic isolates were analysed. Majority were from wound specimens 43.8% (n=1138), followed by abdominal, urogenital and diabetic foot infections with 20.3% (n=528), 6.5% (n=168) and 5.2% (n=136), respectively. Isolates from positive blood cultures represented 3.1% (n=81). Anaerobic Gram-negative bacilli (AGNB) represented 56.5% (n=1470) of isolates; *Bacteroides fragilis* group, *Prevotella* spp. and *Fusobacterium* spp. isolates were present in 33.5% (n=872), 15.2% (n=395) and 7.3% (n=191), respectively. *B. fragilis* was single most predominant species with 11.7% (n=303) isolates. Gram-positive anaerobic cocci (GPAC) represented 23.1% (n=601) of isolates; *Finegoldia magna*, *Peptoniphilus* spp. and *Anaerococcus* spp. were present in 7.7% (n=200), 7.3% (n=189) and 6.2% (n=160), respectively. Finally, Gram-positive anaerobic bacilli (GPAB) represented 17.7% (n=459) of isolates; *Propionibacterium* spp. 6.8% (n=177), *Clostridium* spp. 6.5% (n=168) and *Actinomyces* spp. 4.4% (n=114). Penicillin, co-amoxiclav, imipenem, metronidazole and clindamycin resistances were as follows: 96.9%, 2.8%, 0.3%, 0.0% and 25.4% (*B. fragilis* group); 52.2%, 0.0%, 0.0%, 0.0% and 33.2% (*Prevotella* spp.); 2.6%, 1.4%, 0.0%, 0.0% and 3.7% (*Fusobacterium* spp.); 50.7%, 1.4%, 0.0%, 0.0%, 2.8% (*Veillonella* spp.); 2.5%, 0.2%, 0.0%, 0.0% and 30.1% (GPAC); 12.4%, 0.0%, 1.2%, 0.0% and 19.5% (*Clostridium* spp.); 1.8%, 0.0%,

0.0%, 98.2% and 15.8% (*Actinomyces* spp.); 0.6%, 0.0%, 0.0%, 100% and 9.6% (*Propionibacterium* spp.), respectively.

Conclusions: Metronidazole, imipenem and co-amoxiclav retain excellent overall activity against anaerobic bacteria without intrinsic resistance. Penicillin is active mainly against Gram-positive anaerobes and fusobacteria. Overall clindamycin resistance of 23.7% and its wide distribution among all groups of anaerobes warrants caution when used as an empiric treatment of anaerobic and mixed infections. Clindamycin resistance of *B. fragilis* group isolates in Slovenia rose from 12% in 2003 to 25% in 2014.