Detection of heteroresistant strains

Introduction

Heteroresistance refers to populations containing a majority of bacteria inhibited by concentrations below the susceptibility breakpoint, together with a small number of microorganisms (1 to 1000 to 1 10.000.000) that are resistant.

Heteroresistance has been described in S. aureus and coagulase negative staphylococci, particularly in MRSA, hVISA, Besides, in Enterococcus faecium (teicoplanin), pneumococci (penicillin), Acinetobacter baumannii (carbapenems and colistin), Helicobacter pylori (metronidazole, clarithromycin) Klebsiella pneumoniae (colistin), Clostridium difficile (metronidazole), Ps aeruginosa (carbapenems, piperacillin + tazobactam) Cryptococcus neoformans (fluconazole) among others.

The most common method used to detect heteroresistance is to see colonies inside the inhibition zones of disks (Neo-sensitabs), containing different antimicrobials. There is no doubt that the phenomenon of heteroresistance may be clinically relevant. In S. aureus, heteroresistance to oxacillin/methicillin is clinically relevant.
The clinical importance of S. aureus isolates heteroresistant to glycopeptides, is under discussion, while the amount of hVISA strains is increasing worldwide. The use of antimicrobials in vivo may select heteroresistant mutants, that later on result in a population stable resistant.

Isolates of A. baumanni heteroresistant to colistin may show stable MICs of more than 8 – 16 ug/ml.
These strains can be selected in vitro and probably can be selected in patients treated with colistin.
Ac baumanni isolates from patients previously treated with colistin, show a higher degree of heteroresistance.

Carbapenem heteroresistance in A. baumannii

Fernandez-Cuenca et al (1) indicates that A. baumanni heteroresistant to carbapenems should be detected in the laboratory, because it is probable that the resistant subpopulations, will be selected in the presence of imipenem or meropenem, resulting in a therapeutic failure. Microdilution and automatic methods (Wider, Vitek etc) cannot detect heteroresistance, because they are using too small inocula. To detect heteroresistance methods of diffusion on agar should be used and the inoculum should never be below to that corresponding to a 0.5 Mc Farland.

Pournaras et al (2) in 2005 describes the spread of isolates heteroresistant to carbapenems, they showed subcolonies present in the clear zone of inhibition. Resistant colonies were retested and again a subpopulation of resistant isolates was grown inside the zone of inhibition. Del Rosario Quintana et al (3) conclude that the automatic system Wider cannot detect the carbapenem heteroresistant strains of A. baumanni. Fernandez F et al (4) tested 30 clinical isolates of A. baumannii and heteroresistance was defined as presence of colonies inside the zones around imipenem disks. Heteroresistant strains showed MICs of 4-16 ug/ml towards imipenem and were associated to the presence of the beta-lactamase OXA-58 gen.

Gomez MC et al (5) tested 44 isolates of A. baumannii and concluded that 84 % of the isolates showing colonies inside the zone of imipenem, also possessed the OXA-58 gen, while none of the isolates without colonies inside the zone, possessed the gen OXA-58. Neou et al (6) tested 142 non-repetitive isolates of A. baumannii. Agar MICs for imipenem were 0.25 to 4 ug/ml. Colonies grown at 8 ug/ml did not show resistance stability when subcultured in drug-free medium. Agar MICs for meropenem were 0.25 to 4 ug/ml. Colonies grown at 8 to 32 ug/ml showed stability to meropenem resistance after 1 week subculture in drug-free medium, but they were susceptible to imipenem. The authors suggest that apparently carbapenem susceptible A. baumanni populations contain an amount of resistant meropenem subpopulations. The implementation of screening techniques to identify heteroresistant isolates is of significant importance.

Observe the presence of subcolonies inside the zone of inhibition of Imipenem and Meropenem Neo-sensitabs

Colistin heteroresistance in A. baumannii

Jian Li et al (7) tested 16 colistin susceptible clinical isolates of A. baumannii by population analysis profiles and by serial passaging with or without exposure to colistin. They demonstrated the presence of heterogeneous colistin-resistant A. baumannii in "colistin susceptible" isolates.
The authors conclude that colistin heteroresistant A. baumannii isolates cannot be discriminated from colistin susceptible by MIC measurements alone.
Colistin heteroresistant A. baumannii may be a preliminary stage that leads to the proliferation of resistant subpopulations upon exposure to colistin.
Hawley et al (8) conclude that the isolates exhibiting heteroresistance is significantly higher among isolates recovered from patients previously treated with colistin
Park et al (9) indicates that high colistin resistance rates in Acinetobacter have been reported from Korean hospitals and that was not due to clonal dissemination, but they arose independently.
Hawley et al (10) identified one colistin-dependent A. baumannii isolate. When plated on Mueller Hinton agar with a Colistin 10 ug disk, the isolate grew heavily immediately around the disk.
The Colistin 10 ug Neo-sensitabs prediffusion method (2 hours + 18/22 hours) will detect both colistin heteroresistant and resistant strains.

Carbapenem and PIP + TAZO Heteroresistance in P. aeruginosa.

The presence of subcolonies inside the carbapenems disk zone indicates that heterogeneous subpopulations with reduced susceptibility to carbapenems may exist in a number of P. aeruginosa strains that appear to be carbapenem susceptible by conventional automated susceptibility methods.
Pournaras et al (11) tested 14 non-repetitive isolates of P. aeruginosa in which a few subcolonies appeared within the zone of inhibition of imipenem and meropenem disks. These isolates represented 27.5 % of the apparently carbapenem susceptible isolates.
Population analysis showed distinct subpopulations that grew in concentrations up to 18 ug/ml imipenem and 12 ug/ml meropenem. The heterogeneous subpopulations retained their resistance levels implying a rather stable expression of resistance. Conventional MIC dilution methods, using the standard 10,000 CFU per spot inoculum may miss carbapenem resistant mutants.
Pournaras et al (12) describe an isolate showing heteroresistance to Piperacillin + tazobactam. The isolate was reported as susceptible by automatic methods and by agar dilution. Nevertheless, the isolate exhibited distinct colonies within the inhibition zone around the piperacillin + tazobactam disk.

Colistin resistant P. aeruginosa

Brannon et al (13) tested 19 colistin-resistant P. aeruginosa isolated from colistin-treated cystic fibrosis patients.
75 % of the colistin resistant CF strains were highly resistant to colistin (MIC > 200 ug/ml) and the remaining were moderately resistant (MIC > 2 ug/ml).
Montero et al (14) tested colistin-resistant P. aeruginosa (CORPA)isolated from 10 patients. In all cases, multidrug-resistant P. aeruginosa susceptible only to colistin and amikacin were isolated, before the emergence of CORPA.9 of the patients had previously received prolonged courses of colistin (mean 40 days).
Detection using Colistin 10 ug Neo-sensitabs and the prediffusion method (2hours + 18/22 hours prediffusion)

Colistin heteroresistant /resistant Klebsiella pneumoniae and carbapenem heteroresistant E. aerogenes

Poudyal et al (15) tested 22 multidrug-resistant clinical isolates of K. pnemoniae.6 isolates were colistin-resistant with MICs >= 32 ug/ml. Colistin heteroresistance was observed in 15 of 16 isolates considered colistin-susceptible. Similar to our recent finding of colistin heteroresistance in A. baumannii, the MIC alone may not provide information to guide treatment, because heteroresistance is not detected by an MIC method.
Antoniadou et al (16) tested 18 colistin-resistant K. pneumoniae isolates from 13 patients over a 16 months period. Most of the isolates possessed ESBLs or metallo-beta-lactamases or both. Selective pressure due to extensive or inadequate colistin use may lead to the emergence of colistin resistance.
Papaioannou et al (17) in a study over 3 years (2005-2008) and almost 5000 isolates, found that 1.5 % of A. baumanni, 16 % of Klebsiella pneumoniae and 4.8 % of P. aeruginosa were colistin resistant.
Gordon et al (19) showed that the Microscan failed to detect heteroresistance to carbapenems in a patient with E. aerogenes bacteremia, while disk diffusion and E-test detected it.
Detection using Colistin 10 ug Neo-sensitabs and the prediffusion method (2hours + 18/22 hours prediffusion) and Imipenem/Meropenem Neo-sensitabs by the diffusion method

Metronidazole heteroresistance in Clostridium difficile

Pelaez et al (18) found that initially metronidazole-resistant C. difficile isolates became susceptible after thawing;
However they presented slow-growing subpopulations within the inhibition zones of the metronidazole disk. The authors conclude that: resistance to metronidazole in toxigenic C difficile is heterogeneous, and prolonged exposure to metronidazole can select for in vitro resistance. We recommend routine performance of the disk diffusion method, with primary fresh C. difficile isolates in order to ensure that metronidazole heteroresistant populations do not go undetected.
Similar heterogeneous metronidazole resistance has been observed in Bacteroides spp and Helicobacter pylori.
Detection using Metronidazole Neo-sensitabs. Observe the presence of colonies inside the zone of inhibition

Vancomycin/Teicoplanin heteroresistance in S. aureus and coagulase negative staphylococci

Is treated separately under the prediffusion method (2 hours + 18/22 hours) prediffusion

Vancomycin/Teicoplanin heteroresistance/resistance in Enterococcus

Is treated separately under the prediffusion method (2 hours + 18/22 hours) prediffusion

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