We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

10

Heteroresistance

Meletis Georgios
Clinical Microbiologist, Research Assistant
Aristotle University of Thessaloniki, School of Medicine
Greece

1. Introduction

The emergence of clinical infection due to methicillin resistant *Staphylococcus aureus* (MRSA) with decreased susceptibility to vancomycin and the frequent isolations in some countries of multidrug-resistant (resistant to three or more classes of antimicrobials), extensively drug resistant (resistant to all but one or two classes) or even pandrug-resistant (resistant to all available classes) Gram negative nosocomial pathogens are causing great concern worldwide. In an era of increasing antimicrobial resistance many recent studies have focused on the heteroresistance phenomenon that is considered to be a precursor stage, which may or may not lead to the emergence of a resistant strain.

The term heteroresistance has been mostly used to describe hetero-vancomycin intermediate Staphylococcus aureus (hVISA) that spontaneously produces VISA cells within its cell population at a frequency of 10^{-6} or above (Neoh et al., 2008). In a broader sense though, heteroresistance may be understood as mixed populations of drug-resistant and drug-sensitive cells in a single clinical specimen or isolate where the proportion of resistant organisms may not be explicable by the natural "background" mutation rate alone (Rinder, 2001); and even more precisely, heteroresistance can be defined as resistance to certain antibiotics expressed by a subset of a microbial population that is generally considered to be susceptible to these antibiotics according to traditional *in-vitro* susceptibility testing (Falagas et al., 2008). The frequency of these resistant organisms is about one sub-clone in every 10^5–10^6 colonies, which roughly equals the normal rate of mutation.

2. Heteroresistance in *Staphylococcus aureus*

S. aureus is the species in which the phenomenon has been reported more often and studied more in detail. Heteroresistance to methicillin (Ryffel et al., 1994) and oxacillin (Cimolai et al., 1997; H. Liu et al., 1990; Wannet, 2002) is dependent on the *mecA* gene that codes for a lower-affinity penicillin-binding protein conferring strain-specific variable resistance from borderline to elevated resistance to beta-lactam antibiotics. It has been shown that in addition to this resistance, chromosomal mutations not related to *mecA* may generate a small proportion of highly methicillin-resistant subclones (Strandén et al., 1996).

The first reports of VISA (Hiramatsu et al., 1997a) and hVISA (Hiramatsu et al., 1997b) were made from Japanese hospitals in 1997 and inspired further research because vancomycin has been the drug of choice for infections due to MRSA. Mu 3 was the first MRSA strain

heteroresistant to vancomycin and it is used since then as a control strain in different methodologies for the detection of vancomycin heteroresistance in MRSA. Several later studies from various parts of the world (Borg et al., 2005; Chesneau et al., 2000; Howe et al., 1999; M.N. Kim et al., 2002; Marchese et al., 2000; Reverdy et al., 2001; Wang et al., 2004) have proved that heteroresistance to vancomycin among MRSA was maybe ignored or underestimated but not absent. Furthermore, since early VISA isolates in the USA were resistant to teicoplanin too (Appelbaum, 2007), and reduced susceptibility (Park et al., 2000) or heterogeneous resistance (Nunes et al., 2007) to this drug were also reported, the term glycopeptide-intermediate *S. aureus* (GISA) has been added to indicate a broader resistance profile.

Reduced vancomycin susceptibility of hVISA and VISA strains is not linked to the *vanA* gene, which causes the high-level vancomycin resistance of certain enterococcal species and has been found rarely in vancomycin-resistant MRSA (VRSA) (Cui et al., 2006; Howden et al., 2008) but is rather a consequence of several phenotypic changes (Rong & Leonard, 2010). Among those suggested, the thickened bacterial cell wall is the most frequently observed (Kim et al., 2000). This cell wall change could probably explain also the cross-heteroresistance between vancomycin and daptomycin- a relatively large molecule that has to access relevant binding regions on the bacterial cell membrane in order to act (Sakoulas et al., 2006).

3. Heteroresistance among Gram negative nosocomial pathogens

Carbapenems are the most effective antimicrobial agents against Gram negative bacteria. Furthermore, they are stable to the hydrolytic activity of extended spectrum beta-lactamases and therefore they have been used widely for treating infections caused by multidrug resistant isolates. The extended use however of these compounds has led to the emergence of carbapenem resistance mechanisms mainly in members of the Enterobacteriaceae family, in *Pseudomonas aeruginosa* and *Acinetobacter baumanii*. Carbapenem heteroresistance among *A. baumanii* strains has been reported in a preliminary (Pournaras et al., 2005) and a complete study (Ikonomidis et al., 2009) from Greece where Pournaras et al. found also meropenem heteroresistant subpopulations in six apparently meropenem-susceptible, carbapenemase (KPC)-producing *Klebsiella pneumoniae* (KPC-KP) clinical isolates (Pournaras et al., 2010).

Colistin is among the last-resort therapy for treating infections caused by carbapenem resistant pathogens but resistance to this drug is emerging in multidrug-resistant Gram negative bacteria (Kontopoulou et al., 2010). Heteroresistance to colistin was initially described among clinical *A. baumannii* (Li et al., 2006; C.H. Tan et al., 2007; Yau et al., 2009) and *Enterobacter cloacae* isolates (Lo-Ten-Foe et al., 2007). Intrestingly, in *A. baumannii*, this heteroresistance was documented to be related to previous colistin therapy (Hawley et al., 2008). Heteroresistance to colistin has been reported in *K. pneumoniae* isolates collected from 16 medical centers in various countries (Poudial et al., 2008) and among carbapenemase-producing *K. pneumoniae* (KPC or VIM-1) regardless prior colistin therapy in a Greek hospital where resistant rates to colistin in carbapenemase-producing *K. pneumoniae* rose from 0% in 2007 to 24.3% in 2009 (Meletis et al., 2011).

4. Heteroresistance in other species

Heteroresistance to glycopeptides is not limited in *S. aureus* but has been described for *Enterococcus faecium* (Alam et al., 2001; Qu et al., 2009) and coagulase-negative staphylococci as well, including *S. epidermidis*, *S. auricularis, S. capitis, S. haemolyticus, S. simulans* and *S.*

warneri (Nunes et al., 2006; Wong et al., 1999). Heteroresistance to penicillin has been reported in *Streptococcus pneumoniae* (Morand & Muhlemann, 2007), heteroresistance to fluconazole and other azoles in *Cryptococcus neoformans* (Mondon et al., 1999; Yamazumi et al., 2003) while metronidazole heteroresistance in *Gardnerella vaginalis* has been blamed for therapeutic failures (Altrichter et al., 1994).

The heteroresistance phenomenon is present even in clinical tuberculosis. It is not rare and not restricted to a particular resistance gene, but is obscured by cultivation as well as by some, culture-independent resistance prediction tests (Rinder et al., 2001). In fact, heteroresistant subpopulations are frequent in *Mycobacterium tuberculosis* although they seem to be well hidden. This observation may be explained by studies showing that acquisition of resistance results in an- at least temporary- decrease in growth rate (Billington et al., 1999; Gillespie et al., 2001).

It will be very interesting to find out if heteroresistance exists even among other species or concerns more antimicrobial agents. It is conceivable that there is more to be found and further research may provide new insight in this field.

5. Detection methods

Detection of heteroresistance is difficult and labor-intensive. Disc diffusion method or commercial automated systems may fail to detect most heteroresistant isolates (Lo-Ten-Foe et al., 2007; Tenover, 1999), and E-test results depend on the medium used (T.Y. Tan & Ng., 2007). Gradient plates, Disc-agar Method, E-test GRD (Glycopeptide Resistance Detection), Vancomycin-containig plates and E-test Macrodilution have all been explored for the detection of hGISA and GISA staphylococci but Population Analyses Profiles remains to date the most reliable method to detect heteroresistant subpopulations in Gram positive as well as in Gram negative bacteria. Growth of resistant subcolonies within a clear E-test inhibition zone and Population Screening Plates can be used for screening purposes, therefore, these two techniques are also described in this chapter.

Various studies used Population Analysis Profiles-Area Under the Curve (PAP-AUC) (Wooton et al., 2001) as the gold standard in order to evaluate the sensitivity and specificity of some of the other methods. Walsh et al. evaluated seven methods in 2001 among which, E-test Macrodilution appeared to perform better (sensitivity 96%, specificity 97%) for the detection of *Staphylococcus* strains with reduced susceptibilities to vancomycin (SRSV) (a term that contains both GISA and hGISA) (Walsh et al., 2001). In 2007, Wootton et al. performed a large-scale multicenter study involving 3 different methods of GISA and hGISA detection (brain-heart infusion agar with 6 mg/L vancomycin (BHIA6V), Mueller-Hinton agar with 5 mg/L teicoplanin (MHA5T), and E-test Macrodilution. Among them, BHIA6V had a very low sensitivity for hGISA (11.47%) whereas MHA5T and E-test Macrodilution performed both well. MHA5T though, gave twice as many false-positive results compared to E-test Macrodilution which moreover, resulted in significantly less variation between laboratories (Wooton et al., 2007). In 2011, Satola et al. evaluated the E-test Macrodilution method, E-test GRD and BHI screen agar plates containing 4 mg/L vancomycin and 16 g/L casein using 0.5 and 2.0 McFarland inocula. E-test Macrodilution was 57% sensitive and 96% specific, E-test GRD was 57% sensitive and 97% specific, and BHI screen agar was 90% sensitive and 95% specific with a 0.5 McFarland inoculum and 100% sensitive and 68% specific with a 2.0 McFarland

inoculums (Satola et al., 2011). In the same year, van Hal et al. screened 458 MRSA bloodstream isolates to determine the most accurate testing strategy to detect the presence of heteroresistance. Compared to PAP-AUC, the sensitivities and specificities of E-test Macrodilution, E-test GRD, vancomycin broth microdilution (using a MIC cutoff of ≥ 2 mg/L), and standard vancomycin E-test (using a MIC cutoff of ≥ 2 mg/L) were 89 and 55%, 71 and 94%, 82 and 97%, and 71 and 94%, respectively (van Hal et al., 2011).

Research for the most effective method to detect SRSV is still going on but reports of heteroresistance even among other clinically important species worldwide highlight the need for the development of a reliable and easy-to-use susceptibility testing method that can be used in the everyday laboratory practice.

5.1 Population analysis profiles

Step 1: Prepare agar plates containing varied antibiotic concentrations, for example 0, 0.5, 1, 2, 3, 4, 5, 6, 8… mg/L.

Step 2: Culture bacteria overnight in tryptone soya broth (TSB).

Step 3: Prepare a cell suspension of approximately 10^8 CFU/ml (OD 0.3 at 578 nm or 2:3 dilution of a 0.5 McFarland suspension).

Step 4: Make 10-fold serial dilutions of the cell suspension and spread 50µl from each dilution onto a complete series of antibiotic-containing plates. (Fig. 1)

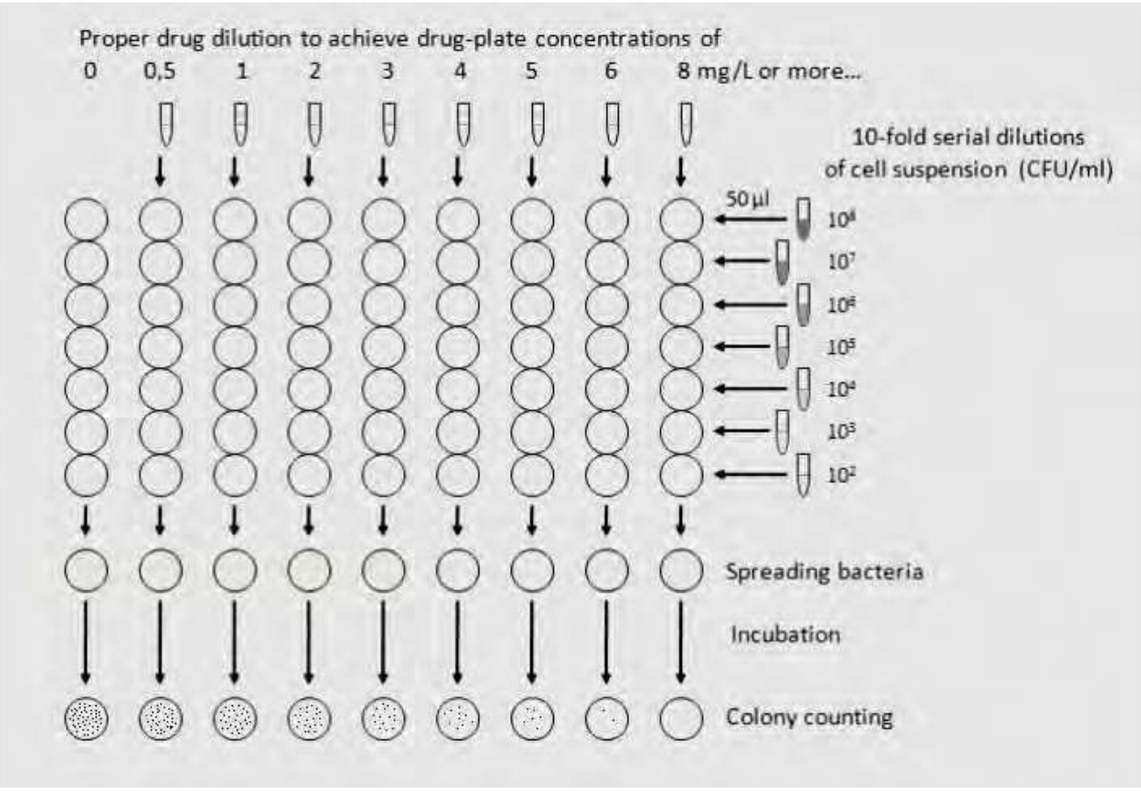


Fig. 1. Meletis, G 2011, 'Population analysis profiles method', in possession of the author.

Step 5: Incubate at 35-37 ^{o}C for 48h.

Step 6: Count the number of colonies grown on each plate (Table 1).

		Drug concentration (mg/L)							
		0,5	1	2	3	4	5	6	8
CFU/ml	10^8								
	10^7								
	10^6								
	10^5								
	10^4								
	10^3								
	10^2								

Table 1. Colony counting table.

Step 7: Plot the colony counts on a semi-logarithmic graph with colony counts on the vertical axis and drug concentration on the horizontal axis (Fig 2).

Step 8: Calculate the frequency of resistant subpopulations at the highest drug concentration by dividing the number of colonies grown on an antibiotic containing plate by the colony counts from the same bacterial inoculum plated onto antibiotic-free plates.

Step 9: Determine the MIC of the resistant subpopulations and reassess it after serial daily subcultures on antibiotic-free medium for 7-14 days in order to evaluate whether the resistance is stable.

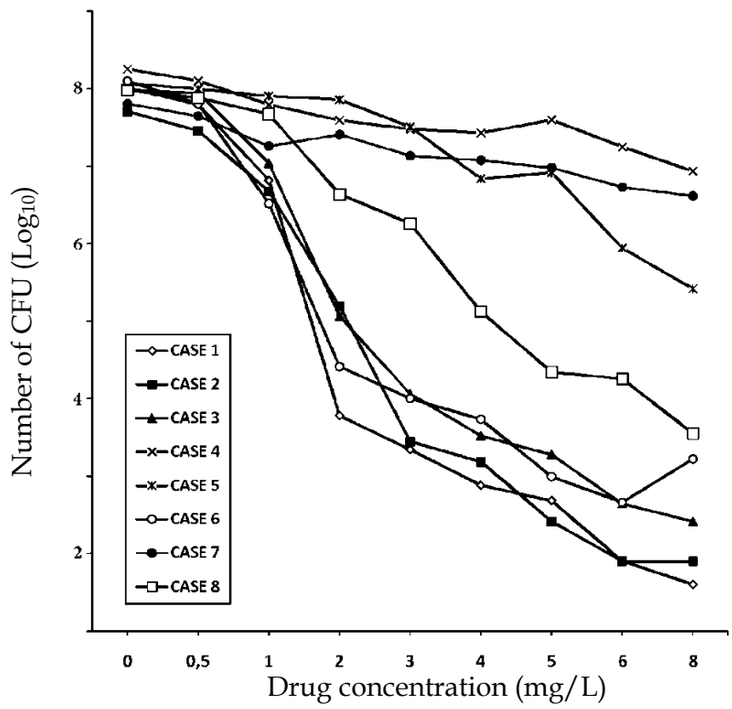


Fig. 2. Colony counts plotting of eight *K. pneumoniae* strains tested for heteroresistance to colistin on a semi-logarithmic graph. Strains 4, 5 and 7 are resistant.

5.2 Modified population analysis profiles-AUC (area under the curve)

Modified PAP-AUC was proposed by Wooton et al. for the detection of heteroresistance to vancomycin among *S. aureus* strains and is considered to date the gold standard for hGISA detection (Wooton et al., 2001). It is based upon the calculation of the ratio of the test strain's AUC divided by the corresponding Mu 3 AUC. After 24h incubation in TSB, cultures are diluted in saline to 10^{-3} and 10^{-6} and plated onto BHIA plates containing 0.5, 1, 2, 2.5 and 4 mg/L vancomycin. Colonies are counted after 48h incubation at 37 ^{o}C and the viable count is plotted against vancomycin concentration. The AUC of the study strain and Mu 3 are calculated and their ratio is evaluated as follows: <0.9, GSSA; 0.9-1.29, hGISA; ≥ 1.3, GISA (Wooton et al., 2005).

5.3 Gradient plates

Step 1: Culture Mu 3 and test strains overnight in TSB.

Step 2: Pour 25 ml of BHIA containing 4 mg/L vancomycin in a 10 cm square Petri dish at an angle of 12^{o} and keep solidified for 30 min in room temperature (Fig. 3).

Step 3: Overlay 25 ml of BHIA and leave to set horizontally. Keep the solidified agar at room temperature for 120 min.

Step 4: Adjust each culture to a turbidity equal to 0.5 McFarland and streak the Mu 3 and test strains onto the gradient agar plate with a cotton swab.

Step 5: Measure the growth distance of the bacteria after 48h incubation at 37 ^{o}C and divide it with the distance grown by Mu 3 on the same plate. A ratio of ≥ 1 denotes an hGISA (Wooton et al., 2001).

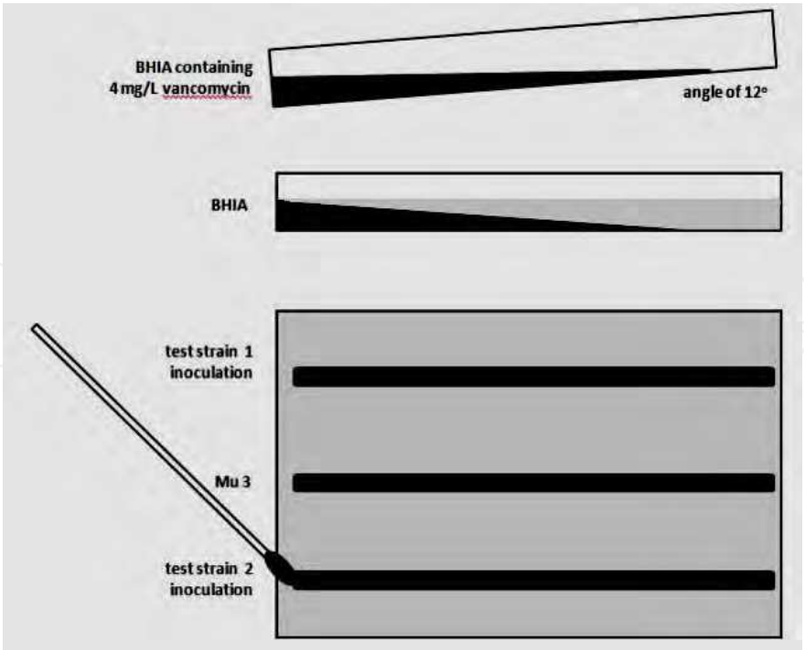


Fig. 3. Meletis, G 2011, 'Gradient plates preparation and inoculation', in possession of the author.

5.4 E-test macrodilution

Bolmström et al. proposed that the use of a dense inoculum, rich medium and a 48h incubation period can optimize the detection of VISA and hVISA (Bolmström et al., 1999).

Step 1: Spread 250 μl of a 2 McFarland suspension onto BHI agar plates and put one vancomycin and one teicoplanin E-test for each strain (Fig. 4).

Step 2: Incubate at 35-37 ^{o}C for 48h.

Step 3: Interpretation (hVISA when vancomycin MIC and teicoplanin MIC ≥ 8 or when teicoplanin MIC ≥ 12 and vancomycin MIC <4).

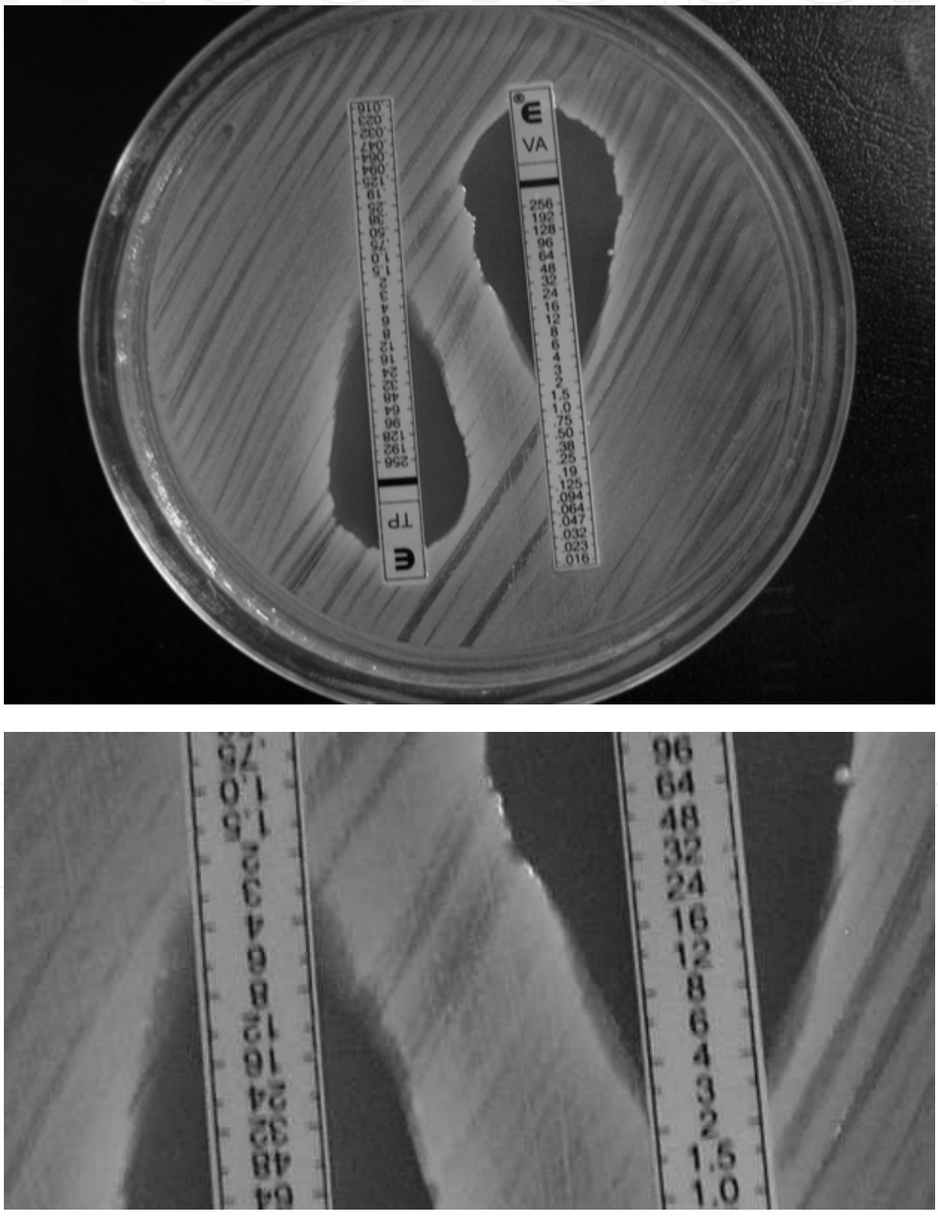


Fig. 4. E-test Macrodilution. Photo courtesy of Dr. Tzampaz Egki.

5.5 Disc-agar method

The disc-agar method has been used to demonstrate both the presence of potential intermediate vancomycin resistant staphylococci and staphylococci with heteroresistance to vancomycin on the same plate. It is based upon the observation that beta-lactam antibiotics (Aritaka et al., 2001; Y.S. Kim et al., 2003) and higher salinity can induce the phenotypic expression of vancomycin resistance and for this purpose aztreonam is preferred over other beta-lactam antibiotics because it has no inhibitory effect on Gram positive bacteria (Fig. 5).

Wong et al. performed a preliminary study of the disc-agar method using different beta-lactams including penicillins, oxacillin, cephalosporin and carbapenems. Although sometimes sattelitism could be seen around other beta-lactam discs, the results were less consistent than they were with aztreonam, and some strains had a large zone of inhibition around the disc (Wong et al., 1999).

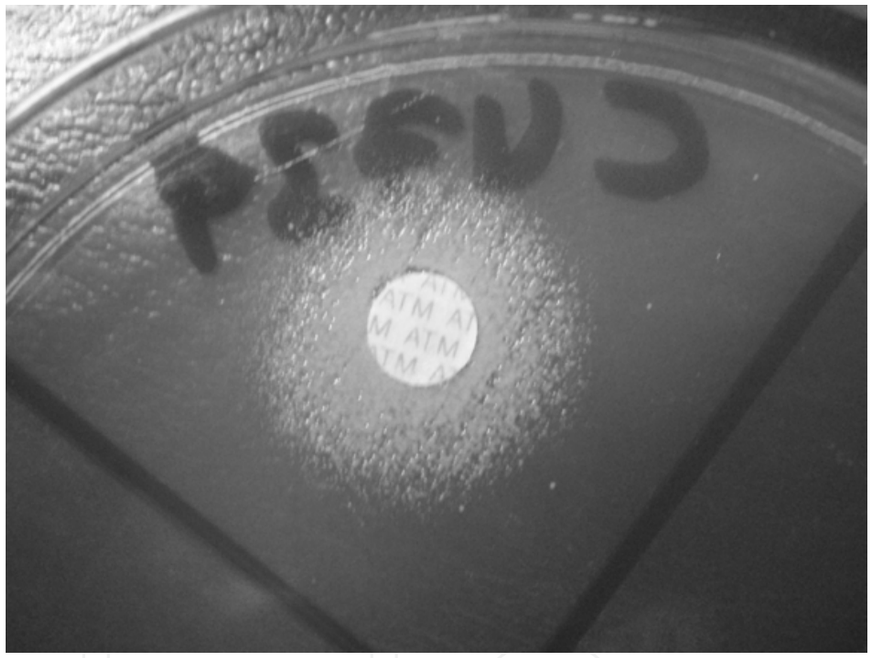


Fig. 5. Bacterial growth around aztreonam disc. Photo courtesy of Dr. Tzampaz Egki.

Step 1: Prepare vancomycin-salt agar plates by incorporating 4 mg/L vancomycin and 4% NaCl into MHA.

Step 2: Make a 1 McFarland bacterial suspension.

Step 3: Inoculate with a sterile cotton swab and place a 30 μg aztreonam disc 5 minutes afterwards.

Step 4: Incubate at 37 ◦C for 48h.

Step 5: Suspect heteroresistance if bacterial growth is observed around the disc. The colonies may be homogeneous or heterogeneous in size (Fig. 6).

Note: In any case further testing by population analysis profiles is recommended for the characterization of the strain's resistance profile.

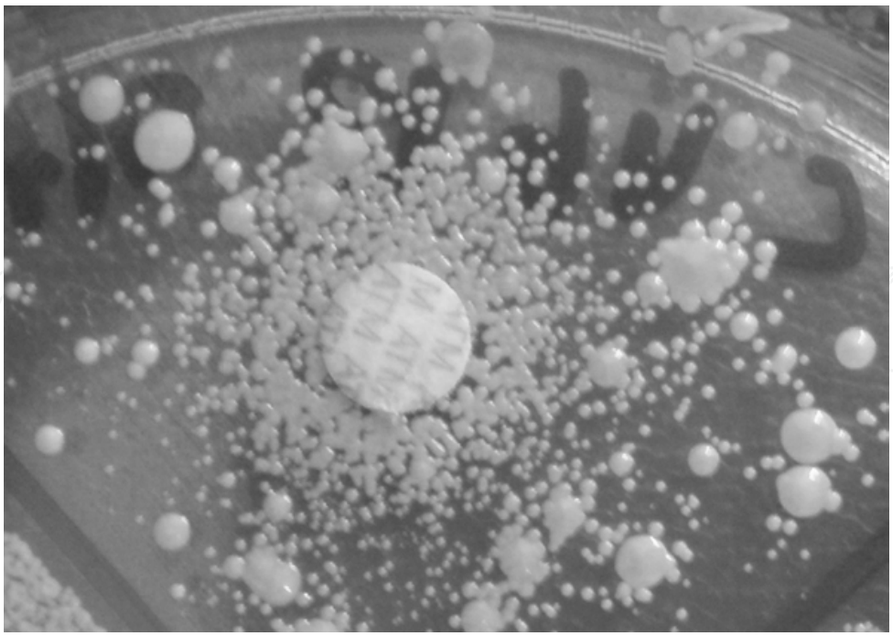


Fig. 6. Different size colonies around aztreonam disc. Photo courtesy of Dr. Tzampaz Egki.

5.6 E-test GRD (glycopeptide resistance detection)

The E-test GRD strip consists of a double-sided gradient of vancomycin and teicoplanin on a calibrated plastic strip. This double-sided predetermined gradient of vancomycin and teicoplanin is used exclusively for the detection of GISA or hGISA phenotypes.

Strains that give a positive E-test GRD result (vancomycin or teicoplanin ≥ 8) should be send to a reference laboratory for further investigation by population analysis profiles (Fig. 7 and 8).

Note: The endpoints read from the E-test GRD strips should not be regarded as true MICs, but rather as modified results with interpretive cutoffs defined for the phenotypic detection of glycopeptides resistance phenotypes in *S. aureus* (Yusof et al., 2007).

Step 1: Determine the MIC of the study strain.

Step 2: Use standard inoculum (0.5 McFarland) onto Mueller Hinton + 5% blood agar plates and add the GRD strip. Ensure that the whole length of the strip is in complete contact with the agar surface. If necessary, remove air pockets by pressing gently on the strip with forceps, always moving from the lowest concentration upwards. Small bubbles under the strip will not affect the results.

Note: Once applied, the strip cannot be moved because the antibiotic is instantaneously released into the agar.

Step 3: Incubation at 35-37 ^{o}C for 24 and 48h.

Step 4: Results interpretation according to manufacturer's instructions (GISA: positive E-test GRD and vancomycin MIC ≥ 4 mg/L, hGISA: positive E-test GRD and vancomycin MIC < 4 mg/L). When mutant colonies are present in the inhibition eclipse, read the result where these colonies are completely inhibited.

Note: Although the procedure is straightforward, proper use of the test requires the judgment of skilled personnel trained in microbiology and antimicrobial susceptibility testing.

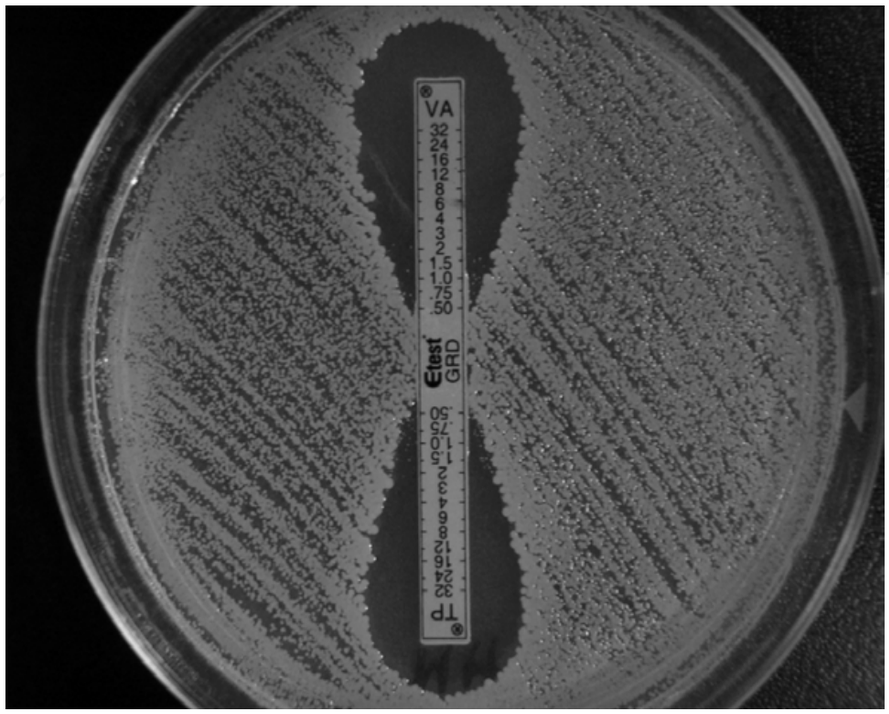


Fig. 7. Negative E-test GRD. Photo courtesy of Dr. Tzampaz Egki.

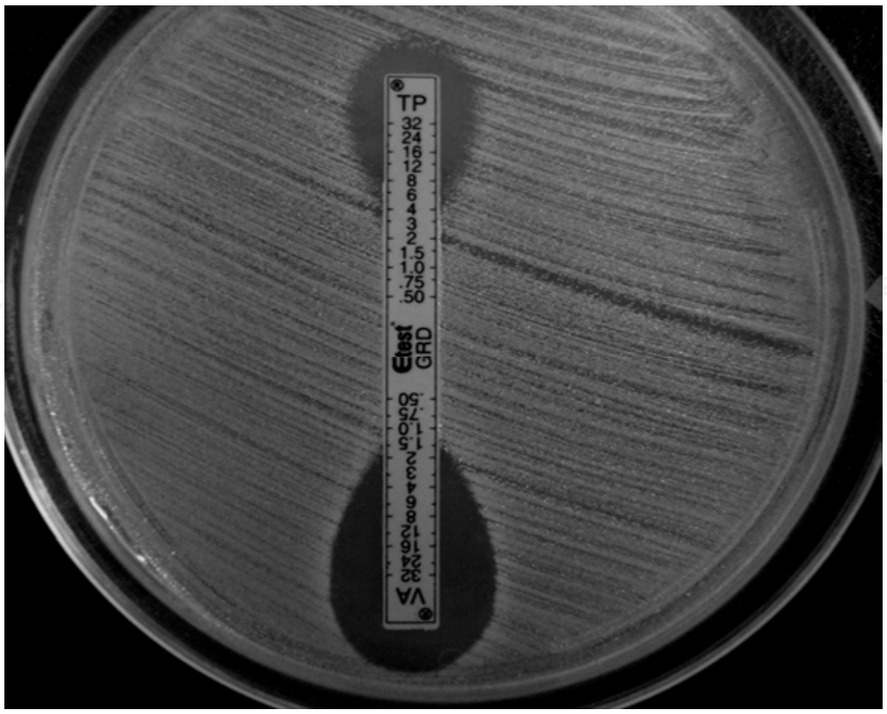


Fig. 8. Positive E-test GRD. Photo courtesy of Dr. Tzampaz Egki.

5.7 Standard E-test

The presence of resistant subcolonies within a clear inhibition zone (Fig. 9) can potentially indicate for a heteroresistant isolate and in such cases, population analysis profiles is suggested.

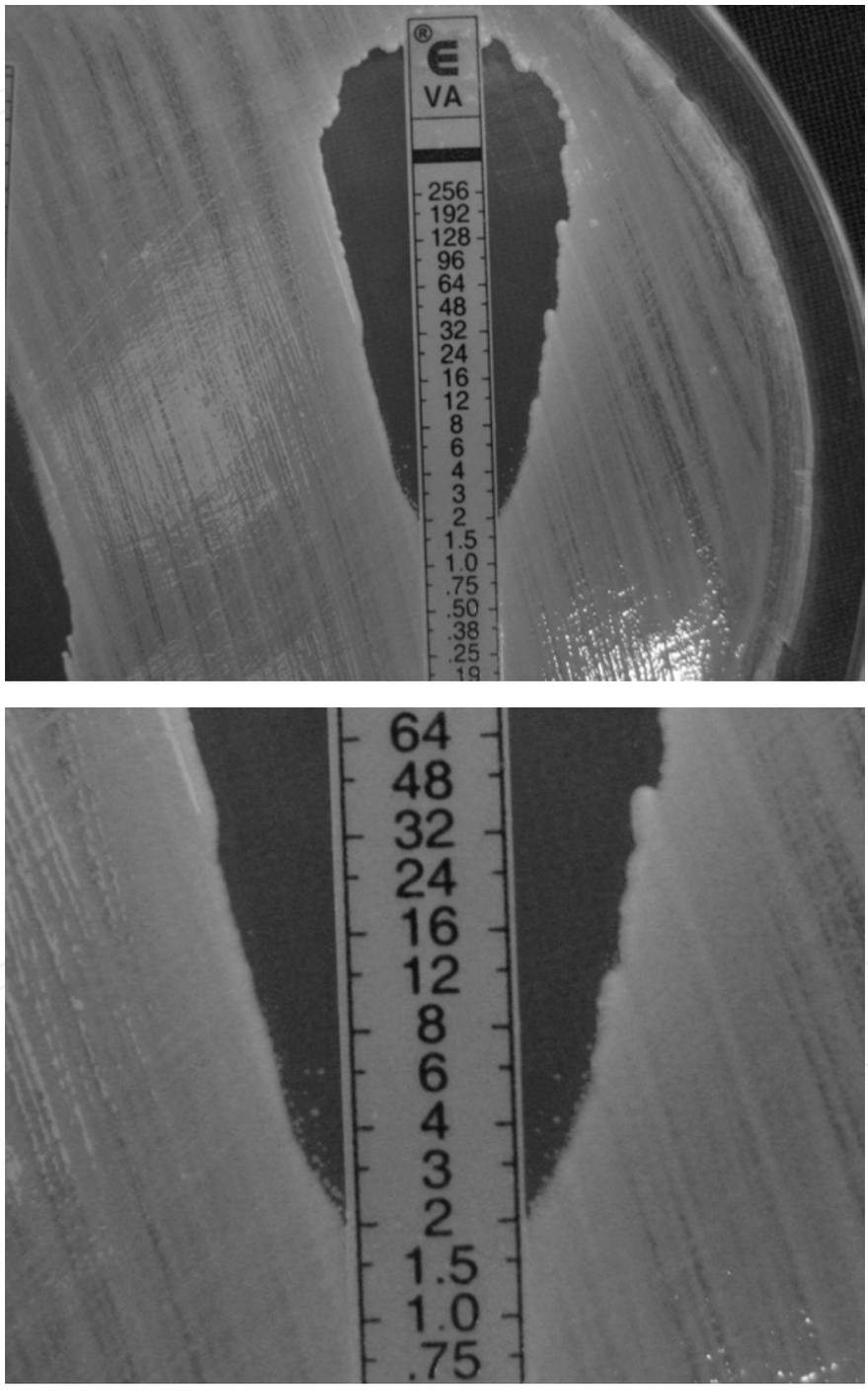


Fig. 9. Subpopulations grown within the inhibition zone. Photo courtesy of Dr. Tzampaz Egki.

5.8 Population screening plates

Screening for potential vancomycin resistance can be performed using BHIA plates with 4 mg/L vancomycin. 10 μl of a bacterial suspension from an overnight culture adjusted to 0.5 McFarland standard is spread on the plate and bacterial growth after incubation at 37 ^{o}C for 24 and 48h is observed. Growth at 24h may be deemed to be of the GISA phenotype and growth occurring between 24 and 48h may be deemed to be an hGISA strain (Walsh et al., 2001).

6. Conclusion

Many studies have tried to associate hGISA with failure of vancomycin therapy but this is still up for debate as results are controversial (van Hal & Paterson, 2011; C. Liu & Chambers., 2003; Neoh et al., 2007; Rong & Leonard, 2010). A further research problem is that such studies are difficult to perform because of the serious nature and high mortality rate among patients infected by MRSA.

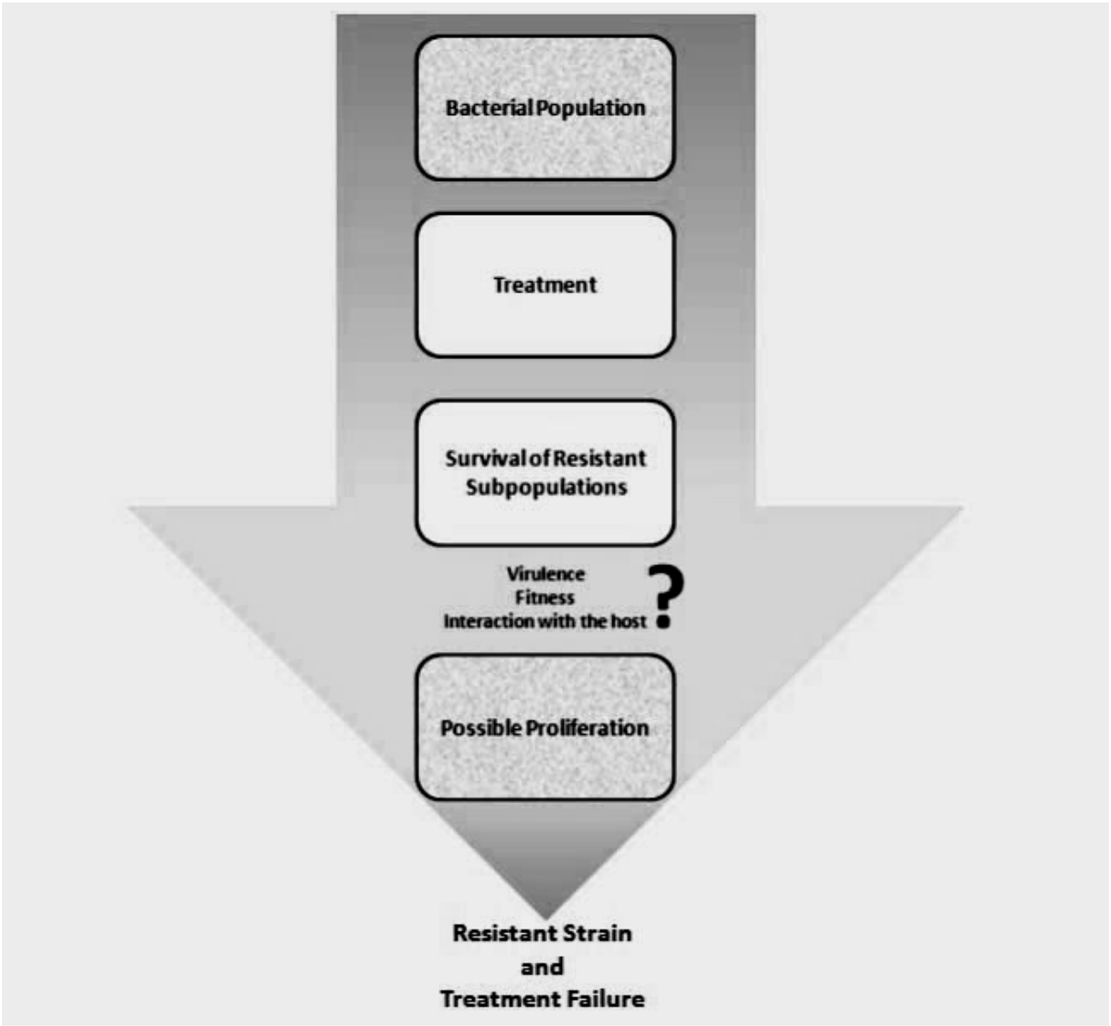


Fig. 10. Meletis, G 2011, 'Natural evolution to drug resistance due to heteroresistance', in possession of the author.

However, heteroresistance could very possibly be a tool for natural evolution to drug resistance, since it provides bacteria with an opportunity to explore the possibility of growth in the presence of antibiotics (Morand & Muhlemann, 2007). The resistant subpopulations may proliferate readily in the absence of competition by the inhibited susceptible cells, thereby giving rise to a new resistant population (Falagas et al., 2008). On the other hand, the emergence of resistant strains *in vitro* may not necessarily have therapeutic implications (Johnson, 1998) mainly because acquisition of resistance in some cases may be accompanied by loss of virulence (Fig. 10).

Nevertheless, heteroresistant strains have been deemed to be responsible for treatment failures (Moore et al., 2003; Wong et al., 1999) and difficulties to detect heteroresistant isolates could already be contributing to a hidden spread of these strains within hospital facilities (Falagas et al., 2008). Therefore, more research is needed to determine the true clinical impact of heteroresistance and its role in the development of fully-resistant bacterial populations.

7. Acknowledgements

The author is most indebted to Dr. Maria Bagkeri, Dr. Egki Tzampaz, Dr. Effrosyni Sianou, and Dr. Danai Sofianou for their help and support in research over the last three years and especially during the study of the heteroresistance phenomenon.

8. References

Alam, M.; Donabedian, S.; Brown, W.; et al. (2001). Heteroresistance to vancomycin in Enterococcus faecium. In: *J Clin Microbiol*, Vol: 39. 3379–81. ISSN: 0095-1137.

Altrichter, T. & Heizmann, W.R. (1994). Gardnerella vaginalis: transport, microscopy, testing resistance. In: *Geburtshilfe Frauenheilkd*, Vol: 54. 606-11. ISSN: 0016-5751.

Appelbaum PC. (2007). Reduced glycopeptide susceptibility in methicillin-resistant Staphylococcus aureus (MRSA). In: *Int J Antimicrob Agents*, Vol: 30. 398-408. ISSN: 0924-8579.

Aritaka, N.; Hanaki, H.; Cui, L.; Hiramatsu, K. (2001). Combination effect of vancomycin and beta-lactams against a Staphylococcus aureus strain, Mu3, with heterogeneous resistance to vancomycin. In: *Antimicrob Agents Chemother*, Vol: 45. 1292–4. ISSN: 0066-4804.

Billington, O.J.; McHugh, T.D.; Gillespie, S.H. (1999). Physiological Cost of Rifampin Resistance Induced In Vitro in Mycobacterium tuberculosis. In: *Antimicrob Agents Chemother,* Vol: 43. 1866-9. ISSN:0066-4804.

Bolmström, A.; Karlsson, A. & Wong, P. (1999). Macro-method conditions are optimal for detection of low level glycopeptides resistance in staphylococci. In: *Clin Microbiol Infect,* Vol: 5(S3). 113. ISSN: 1198-743X.

Borg, M.A.; Zerafa, R.; Morrison, D.; et al. (2005). Incidence of glycopeptide hetero-intermediate Staphylococcus aureus strains in Maltese hospitals. In: *Clin Microbiol Infect,* Vol: 11. 405-7.ISSN: 1198-743X.

Chesneau, O.; Morvan, A. & Solh, N.E. (2000). Retrospective screening for heterogeneous vancomycin resistance in diverse Staphylococcus aureus clones disseminated in French hospitals. In: *J Antimicrob Chemother,* Vol:45. 887-90. ISSN: 0305-7453.

Cimolai, N.; Trombley, C. & Zaher, A. (1997). Oxacillin susceptibility of coagulase-negative staphylococci: role for mecA genotyping and E-test susceptibility testing. In: *Int J Antimicrob Agents,* Vol: 8. 121-5. ISSN: 0924-8579.

Cui, L.; Iwamoto, A.; Lian, J.Q.; et al. (2006). Novel mechanism of antibiotic resistance originating in vancomycin-intermediate Staphylococcus aureus. In: *Antimicrob Agents Chemother,* Vol: 50. 428-38. ISSN: 0066-4804.

Falagas, M.E.; Makris, G.C.; Dimopoulos, G.; et al. (2008).Heteroresistance: a concern of increasing clinical significance? In: *Clin Microbiol Infect,* Vol: 14. 101–4. ISSN: 1198-743X.

Gillespie, S.H. (2001). Antibiotic resistance in the absence of selective pressure. In: *Int J Antimicrob Agents,* Vol: 17. 171-6. ISSN: 0924-8579.

van Hal, S.J.; Wehrhahn, M.C.; Barbagiannakos, T.; et al. (2011). Performance of various testing methodologies for detection of heteroresistant vancomycin-intermediate Staphylococcus aureus in bloodstream isolates. In: *J Clin Microbiol,* Vol: 49. 1489-94. ISSN: 0095-1137.

van Hal, S.J. & Paterson, D.L. (2011). Systematic Review and Meta-Analysis of the Significance of Heterogeneous Vancomycin-Intermediate Staphylococcus aureus Isolates. In: *Antimicrob Agents Chemother,* Vol: 55. 405-10. ISSN: 0066-4804.

Hawley, J.S.; Murray, C.K, & Jorgensen, J.H. (2008). Colistin heteroresistance in Acinetobacter and its association with previous colistin therapy. In: *Antimicrob Agents Chemother,* Vol: 52. 351-2. ISSN: 0066-4804.

Hiramatsu, K.; Hanaki, H.; Ino, T.; et al. (1997a). Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. In: *J Antimicrob Chemother,* Vol: 40. 135–6. ISSN: 0305-7453.

Hiramatsu, K.; Aritaka, N.; Hanaki, H.; et al. (1997b). Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to vancomycin. In: *Lancet,* Vol: 350. 1670–3. ISSN: 0140-6736.

Howden, B.P.; Stinear, T.P.; Allen, D.L.; et al. (2008). Genomic analysis reveals a point mutation in the two-component sensor gene graS that leads to intermediate vancomycin resistance in clinical Staphylococcus aureus. In: *Antimicrob Agents Chemother,* Vol: 52. 3755-62. ISSN: 0066-4804.

Howe, R.A.; Wooten, M.; Walsh, T.R., et al. (1999). Expression and detection of hetero-vancomycin resistance in Staphylococcus aureus. In: *J Antimicrob Chemother,* Vol: 44. 675–8. ISSN: 0305-7453.

Ikonomidis, A.; Neou, E.; Gogou, V.; et al. (2009). Heteroresistance to Meropenem in Carbapenem-Susceptible Acinetobacter baumannii. In: *J Clin Microbiol,* Vol: 47. 4055-9. ISSN: 0095-1137.

Johnson, AP. (1998). Intermediate vancomycin resistance in Staphylococcus aureus: a major threat or a minor inconvenience? In: *J Antimicrob Chemother,* Vol: 42. 289-91. ISSN: 0305-7453.

Kim, M.N.; Pai, C.H.; Woo, J.H.; et al. (2000). Vancomycin-intermediate Staphylococcus aureus in Korea. *J Clin Microbiol,* Vol: 38. 3879-81. ISSN: 0095-1137.

Kim, M.N.; Hwang, S.H.; Pyo, Y.J.; et al. (2002). Clonal spread of Staphylococcus aureus heterogeneously resistant to vancomycin in a university hospital in Korea. In: *J Clin Microbiol,* Vol: 40. 1376–80. ISSN: 0095-1137.

Kim, Y.S.; Kiem, S.; Yun, H.J.; et al. (2003). Efficacy of vancomycin-β-lactam combinations against Heterogeneously Vancomycin-Resistant Staphylococcus aureus (hetero-VRSA). In: *J Korean Med Sci,* Vol: 18. 319-24. ISSN: 1011-8934

Kontopoulou, K.; Protonotariou, E.; Vasilakos, K.; et al. (2010). Hospital outbreak caused by Klebsiella pneumoniae producing KPC-2 b-lactamase resistant to colistin. In: *J Hosp Infect,* Vol: 76. 70–3.ISSN: 0195-6701.

Li, J.; Rayner, C.R.; Nation, R.L.; et al. (2006). Heteroresistance to colistin in multidrug-resistant Acinetobacter baumannii. In: *Antimicrob Agents Chemother,* Vol: 50. 2946-50. ISSN: 0066-4804.

Liu, C. & Chambers, H.F. (2003). Staphylococcus aureus with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. In: *Antimicrob Agents Chemother,* Vol: 47. 3040–5. ISSN: 0066-4804.

Liu, H.; Buescher, G.; Lewis, N.; et al. (1990). Detection of borderline oxacillin-resistant Staphylococcus aureus and differentiation from methicillin-resistant strains. In: *Eur J Clin Microbiol Infect Dis,* Vol: 9. 717-24. ISSN: 0934-9723.

Lo-Ten-Foe, J.R.; de Smet, A.M.; Diederen, B.M.; et al. (2007). Comparative evaluation of the VITEK 2, disk diffusion, etest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant Enterobacter cloacae and Acinetobacter baumannii strains. In: *Antimicrob Agents Chemother,* Vol: 51. 3726-30. ISSN: 0066-4804.

Marchese, A.; Balistreri, G.; Tonoli, E.; et al. (2000). Heterogeneous vancomycin resistance in methicillin resistant Staphylococcus aureus strains isolated in a large Italian hospital. In: *J Clin Microbiol,* Vol: 38. 866–9. ISSN: 0095-1137.

Meletis, G.; Tzampaz, E.; Sianou, E.; et al. (2011). Colistin heteroresistance in carbapenemase-producing Klebsiella pneumoniae. In: *J Antimicrob Chemother,* Vol: 66. 946-947. ISSN: 0305-7453.

Mondon, P.; Petter, R.; Amalfitano, G.; et al. (1999). Heteroresistance to Fluconazole and Voriconazole in Cryptococcus neoformans. In: *Antimicrob Agents Chemother,* Vol: 43. 1856-61. ISSN: 0066-4804.

Moore, M.R.; Perdreau-Remington, F. & Chambers, H.F. (2003). Vancomycin treatment failure associated with heterogeneous vancomycin-intermediate Staphylococcus aureus in a patient with endocarditis and in the rabbit model of endocarditis. In: *Antimicrob Agents Chemother,* Vol:47. 1262–6. ISSN: 0066-4804.

Morand, B. & Muhlemann, K. (2007). Heteroresistance to penicillin in Streptococcus pneumonia. In: *Proc Natl Acad Sci USA,* Vol: 104. 14098–103. ISSN: 1091-6490.

Neoh, H.; Hori, S.; Komatsu, M.; et al. (2007). Impact of reduced vancomycin susceptibility on the therapeutic outcome of MRSA bloodstream infections. In: *Annals of Clinical Microbiology and Antimicrobials,* Vol: 6. 13. ISSN: 1476-0711.

Neoh, H.; Cui, L.; Yuzawa, H.; et al. (2008). Mutated Response Regulator graR Is Responsible for Phenotypic Conversion of Staphylococcus aureus from Heterogeneous Vancomycin-Intermediate Resistance to Vancomycin-Intermediate Resistance. In: *Antimicrob Agents Chemother,* Vol: 52. 45-53. ISSN: 0066-4804.

Nunes, A.; Teixeira, L.M.; Pontes Iorio, N.L.; et al. (2006). Heterogeneous resistance to vancomycin in Staphylococcus epidermidis, Staphylococcus haemolyticus and Staphylococcus warneri clinical strains: characterisation of glycopeptide susceptibility profiles and cell wall thickening. In: *Int J Antimicrob Agents,* Vol: 27. 307–15. ISSN: 0924-8579.

Nunes, A.; Schuenck, R.P.; Bastos, C.R.; et al. (2007). Heterogeneous resistance to vancomycin and Teicoplanin Among Staphylococcus spp. Isolated from Bacteremia. In: *Brazilian J Infect Dis,* Vol: 11. 345-50. ISSN: 1413-8670.

Park, Y.J.; Kim, M.; Oh, E.J.; et al. (2000). Screening method for detecting staphylococci with reduced susceptibility to teicoplanin. In: *J Microbiol Methods,* Vol: 40. 193-8. ISSN: 0167-7012.

Poudyal, A.; Howden, P.B.; Bell, J.M.; et al. (2008). In vitro pharmacodynamics of colistin against multidrug-resistant Klebsiella pneumoniae. In: *J Antimicrob Chemother,* Vol: 62. 1311–8. ISSN: 0305-7453.

Pournaras, S.; Ikonomidis, A.; Markogiannakis, A.; et al. (2005). Heteroresistence to carbapenems in Acinetobacter baumannii. In: *J Antimicrob Chemother,* Vol: 55. 1055–6. ISSN: 0305-7453.

Pournaras, S.; Kristo, I.; Vrioni, G.; et al. (2010). Characteristics of Meropenem Heteroresistance in Klebsiella pneumonia Carbapenemase (KPC)-Producing Clinical Isolates of K. pneumoniae. In: *J Clin Microbiol,* Vol: 48. 2601-4. ISSN: 0095-1137.

Qu, T.; Zhang, J.; Zhou, Z.; et al. (2009). Heteroresistance to Teicoplanin in Enterococcus faecium Harboring the vanA Gene. In: *J Clin Microbiol,* Vol: 47. 4194-6. ISSN: 0095-1137.

Reverdy, M.E.; Jarraud, S.; Bobin-Dubreux, S.; et al. (2001). Incidence of Staphylococcus aureus with reduced susceptibility to glycopeptides in two French hospitals. In: *Clin Microbiol Infect,* Vol: 7.267-72. ISSN: 1198-743X.

Rinder, H. (2001). Hetero-resistance: an under-recognised confounder in diagnosis and therapy? In: *J Med Microbiol,* Vol: 50. 1018-20. ISSN: 0022-2615.

Rinder, H.; Mieskes, K.T. & Loscher, T. (2001). Heteroresistance in Mycobacterium tuberculosis. In: *Int J Tuberc Lung Dis,* Vol: 5. 339–45. ISSN: 1027-3719.

Rong, S.L. & Leonard, S.N. (2010). Heterogeneous vancomycin resistance in Staphylococcus aureus: a review of epidemiology, diagnosis, and clinical significance. In: *Ann Pharmacother,* Vol: 44. 844-50. ISSN: 1060-0280.

Ryffel, C.; Strassle, A.; Kayser, F.H.; et al. (1994). Mechanisms of Heteroresistance in Methicillin-Resistant Staphylococcus aureus. In: *Antimicrob Agents Chemother,* Vol: 38. 724-8. ISSN:0066-4804.

Sakoulas, G.; Alder, J.; Thauvin-Eliopoulos, C.; et al. (2006). Induction of daptomycin heterogeneous susceptibility in Staphylococcus aureus by exposure to vancomycin. In: *Antimicrob Agents Chemother,* Vol: 50. 1581–5. ISSN: 0066-4804.

Satola, S.W.; Farley, M.M.; Andrerson, K.F.; et al. (2011). Comparison of Detection Methods for Heteroresistant Vancomycin-Intermediate Staphylococcus aureus, with the Population Analysis Profile Method as the Reference Method. In: *J Clin Microbiol,* Vol: 49. 177-83. ISSN: 0095-1137.

Strandén, A.M.; Roos, M. & Berger-Bächi, B. (1996). Glutamine synthetase and heteroresistance in methicillin-resistant Staphylococcus aureus. In: *Microb Drug Resist,* Vol: 2. 201-7. ISSN: 1076-6294.

Tan, C.H.; Li, J. & Nation, R.L. (2007). Activity of colistin against heteroresistant Acinetobacter baumannii and emergence of resistance in an in vitro pharmacokinetic/pharmacodynamic model. In: *Antimicrob Agents Chemother,* Vol: 51. 3413-5. ISSN: 0066-4804.

Tan, T.Y. & Ng, S.Y. (2007). Comparison of Etest, Vitek and agar dilution for susceptibility testing of colistin. In: *Clin Microbiol Infect,* Vol: 13. 541–4. ISSN: 1198-743X.

Tenover, F.C. (1999). Implications of vancomycin-resistant Staphylococcus aureus. In: *J Hosp Infect,* Vol:43 Suppl: S3-7. ISSN: 0195-6701.

Walsh, T.R.; Bolmström, A.; Quarnstrom, A.; et al. (2001). Evaluation of current methods of detecting vancomycin resistance and hetero-resistance in Staphylococcus aureus and other staphylococci. In: *J Clin Microbiol,* Vol: 39. 2439–44. ISSN: 0095-1137.

Wang, J.L.; Tseng, S.P.; Hsueh, P.R.; et al. (2004).Vancomycin heteroresistance in methicillin-resistant Staphylococcus aureus, Taiwan. In: *Emerg Infect Dis,* Vol: 10. 1702-4. ISSN: 1080-6040.

Wannet, W. (2002). Spread of an MRSA clone with heteroresistance to oxacillin in the Netherlands. In: *Eurosurveillance* Vol: 5. 73–4. ISSN: 1560-7917.

Wong, S.; Ho, P.L.; Woo, P.C.; et al. (1999). Bacteremia caused by staphylococci with inducible vancomycin heteroresistance. In: *Clin Infect Dis,* Vol: 29. 760–7. ISSN: 1058-4838.

Wootton, M.; Howe, R.A.; Hillman, R.; et al. (2001). A modified population analysis method (PAP) to detect heteroresistance to vancomycin in Staphylococcus aureus in a UK hospital. In: *J Antimicrob Chemother,* Vol: 47. 399–403. ISSN: 0305-7453.

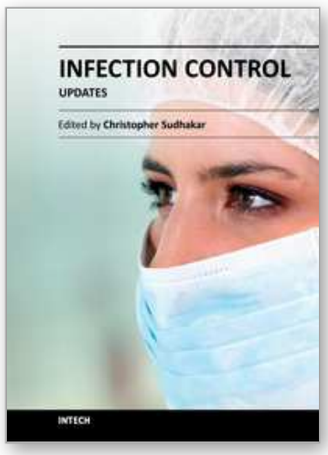
Wootton, M.; Bennett, P.M.; MacGowan, A.P.; et al. (2005). Reduced expression of the atl autolysin gene and susceptibility to autolysis in clinical heterogeneous glycopeptide-intermediate Staphylococcus aureus (hGISA) and GISA strains. In: *J Antimicrob Chemother,* Vol: 56: 944–7.ISSN: 0305-7453.

Wooton, M.; MacGowan, A.P.; Walsh, T.R.; et al. (2007). A Multicenter Study Evaluating the Current Strategies for Isolating Staphylococcus aureus Strains with Reduced Susceptibility to Glycopeptides. In: *J Clin Microbiol,* Vol: 45. 329-32. ISSN: 0095-1137.

Yamazumi, T.; Pfaller, M.A.; Messer, S.A.; et al. (2003). Characterization of heteroresistance to fluconazole among clinical isolates of Cryptococcus neoformans. In: *J Clin Microbiol,* Vol: 41. 267–72. ISSN: 0095-1137.

Yau, W.; Owen, R.J.; Poudyal, A.; et al. (2009). Colistin hetero-resistance in multidrug-resistant Acinetobacter baumannii clinical isolates from the Western Pacific region in the SENTRY antimicrobial surveillance programme. In: *J Inf,* Vol: 58. 138-44. ISSN: 0163-4453.

Yusof, A.; Engelhardt, A.; Karlsson, A.; et al. (2008). Evaluation of a New Etest Vancomycin-Teicoplanin Strip for Detection of Glycopeptide-Intermediate Staphylococcus aureus (GISA), in Particular, Heterogeneous GISA. In: *J Clin Microbiol,* Vol: 46. 3042-7. ISSN: 0095-1137.



Infection Control - Updates

Edited by Dr. Christopher Sudhakar

ISBN 978-953-51-0055-3
Hard cover, 198 pages
Publisher InTech
Published online 22, February, 2012
Published in print edition February, 2012

Health care associated infection is coupled with significant morbidity and mortality. Prevention and control of infection is indispensable part of health care delivery system. Knowledge of Preventing HAI can help health care providers to make informed and therapeutic decisions thereby prevent or reduce these infections. Infection control is continuously evolving science that is constantly being updated and enhanced. The book will be very useful for all health care professionals to combat with health care associated infections.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Meletis Georgios (2012). Heteroresistance, Infection Control - Updates, Dr. Christopher Sudhakar (Ed.), ISBN: 978-953-51-0055-3, InTech, Available from: http://www.intechopen.com/books/infection-control-updates/heteroresistance

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen