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ORIGINAL ARTICLE

Automated Vitek-2 System versus D Test in Detection of Inducible Clindamycin Resistance *Staphylococcus aureus*

Heba-Allah F. EL-Marakby*, Ahmed S. Osman, Emad A. Basyoni and Reem R. Abd El-Galil
Department of Medical Microbiology and Immunology, Faculty of Medicine - Benha University

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****Corresponding Author:***
Heba-Allah F. EL-Marakby
Dept. of Microbiology & Immunology
Tel.: +002-01003659729
heba.fo2ad@yahoo.com

ABSTRACT

Background: *Macrolide, lincosamide and streptogramin type B (MLSB) antibiotics have great importance in the treatment of Staphylococcus aureus infections and existence of isolates that have the ability to resist against MLSB antibiotics is troublesome. Routine testing of staphylococcal isolates for inducible clindamycin resistance (ICR) is supported by the Clinical Laboratory Standards Institute (CLSI). Automated system Vitek 2 offers a schedule that reveals inducible clindamycin resistance directly. it is simple and more cost-efficacious than the more terrible CLSI reference methods.* ***Objectives****: Evaluation the utility of automated vitek-2 system in detection of inducible clindamycin resistance S. aureus compared to D zone test and detection the predominance of erm A and erm C genes among isolated strains.* ***Methodology:*** *25 clinical staphylococcus aureus isolates (Erythromycin intermediate and resistant, Clindamycin susceptible) were examined for ICR both by D- test and Vitek-2 system. multiplex PCR was carried out for the isolates to reveal ermA and ermC genes.* ***Results****: Out of the 25 isolates, ICR was detected by Vitek-2 in 11 isolates (44%). Two of the isolates were not detected by Vitek-2 but confirmed by D-test. Sensitivity, specificity, positive and negative predictive values were calculated as 85.7%, 100%, 100% and 84.6% respectively. erm C and A genes were detected in (40%) and (24%) of the studied isolates respectively. Both C and A genes were detected in (12%).* ***Conclusion****: Vitek-2 is considered a potentially reliable test for detection of ICR, further studies are advised on considerable number of isolates.*

INTRODUCTION

Methicillin-resistant *Staph aureus* (MRSA) were recognized as the most important pathogens that were frequently isolated, and caused serious and life threatening clinical infections such as nosocomial and community-acquired infections. Vancomycin and teicoplanin are usually used to treat the infections with MRSA, however,lately isolation of *S. aureus* with reduce susceptibility or resistance to glycopeptides impetus the physicians to prescribe of other alternative treatments such as Macrolide - Lincosamide - Streptogramin (MLS) .[1]

Clindamycin is often utilized for the therapy of skin, soft tissue, bone and joint infections caused by *S. aureus* because of its oral bioavailability is high and has a great tissue penetration. It is less expensive with little side effects than other anti-staphylococcal agents as vancomycin, linezolid and daptomycin, and may provide additional benefit by inhibiting toxin production due to its mechanism of action (inhibition of protein synthesis by binding to the 50S subunit of the bacterial ribosome).[2]

In this condition, clindamycin is counted to be a good option, due to its action against biofilm formation and bacterial adherence, an elevated level of bone and joint penetration, and a good tolerance. Also, clindamycin motivates bacterial resistance only slowly.[3]

Mechanisms of resistance to MLS antibiotics among staphylococci including: an active efflux pump encoded by *msrA* gene (cause resistance to macrolids and type B streptogramins, and not to clindamycin, Enzymatic inactivation of antibiotic and ribosomal target modification that is the main mechanism of resistance and affects macrolides, lincosamides, and type B streptogramins (MLS resistance). In staphylococci, the four genes, ermA, *ermB*, *ermC* and *ermF*, are frequently involved in resistance to MLS. [1]

MLSB phenotypes in *S. aureus* include three, a constitutive resistant phenotype (cMLSB), a clindamycin-susceptible phenotype in vitro with inducible resistance in vivo (iMLSB), and a clindamycin-susceptible and macrolide-steptogramin B-resistant phenotype (MSB). [4]

Inducible resistance happened when the inactive mRNA produced by the production of methylases becomes active in the presence of an inducer, while in constitutive resistance strains active methylase mRNA is produced. The strains carrying the inducible *erm*

gene are resistant to the inducer and still susceptible to non-inducer macrolides and lincosamides. [5]

D-zone test is a simple and easy test that can discriminate between staphylococci that have inducible *erm* genes-mediated resistance and those which have efflux pump-mediated resistance [6].

In case of testing an iMLSB resistance strain according to Clinical and Laboratory Standards Institute (CLSI) methods with a 15- mg erythromycin disk placed close to a 2- mg clindamycin disk, the zone of inhibition around the clindamycin disk is flattened to form a "D" shape (positive D-test), but in the case of MS phenotype, the clindamycin zone remains circular[7].

Commercial automated systems for identification and susceptibility testing of bacteria are now found in most clinical microbiology laboratories in the United States. Because of their ease, simple use and cost-effectiveness they become the preferred methods over the more complicated (CLSI) reference methods of broth micro-dilution (BMD) and disk diffusion (DD) [8].

The automated system Vitek 2 (bioMérieux, Marcy l'É toile, France) now presents a panel that detects inducible clindamycin resistance immediately.[9]

The aim of this work is evaluation of the utility of automated vitek-2 system in detection of inducible clindamycin resistance *S. aureus* compared to D zone test and finding out the prevalence of *erm* A and *erm* C genes among isolated strains.

METHODOLOGY

This work was done at Microbiology and Immunology Department, Benha Faculty of Medicine from May2017 to November 2017.

The study was performed on 25 *S. aureus* isolates (Erythromycin intermediate and resistant. Clindamycin susceptible) from 60 different clinical samples (abscess, burns, wounds and respiratory secretions). These patients were attending wards of Intensive Care Unit (ICU), chest, and general surgery Departments of Benha University Hospitals.

- **Antibiotic susceptibility and antibiogram:**

Antibiotic susceptibility was carried out for all isolates by using a sterile swab, the plates of Mueller Hinton agar were inoculated after dipping the swab into 0.5 McFarland equivalent bacterial suspension. Using sterile forceps, the antibiotic discs were placed in the center of the M.H agar plates and pressed gently to ensure good contact. The discs were Cefoxtin (FOX=30µg), Amoxaciilin (AX=25µg), Vancomycin (VA=30µg), Ceftazidime (CAZ=30µg), Ofloxcin (OFX=5µg), Cefotaxime (CTX=30µg), Erythromycin (E=15µg) and Clindamycin (DA=2µg).

- **Detection of inducible MLSB resistance (D test):**

A bacterial suspension equal to 0.5 McFarland was prepared for the organisms and was inoculated onto a Mueller–Hinton agar (MHA). Clin (2 µg) and Ery (15 µg) discs were placed15-21 mm edge to edge on the MHA. Plates were analyzed after 24 h of incubation at 35°C. When the Ery zone is ≤22 mm and the Clin zone is 21 mm with a D-shaped zone around the CLin, the organism is positive for inducible resistance (D-test positive).[10]

Antibiotic susceptibility by Vitek- 2 system:

The AST card for VITEK- 2 Systems is an automated test methodology based on the MIC technique reported by MacLowry and Marsh and Gerlach. The organism suspension to be tested must be diluted to a standardized concentration in 0.45% saline before being used for the rehydration of the antimicrobial medium within the card. The card was then filled, sealed, and placed into the instrument incubator/reader VITEK- 2 system. The instrument monitored the growth of each well in the card within a defined period of time (up to 18 hours for bacteria). At the end of the incubation cycle, MIC values were determined for each type of antimicrobial on the card. [11]. The card that used in this study was AST-GP67 (BioMerieux ,France).

Genotypic identification of ICR *S.aureus*:

DNA extraction:

Extraction was done using Zymo Researsh (ZR) Fungal/Bacterial DNA MiniPrep™ Catalog No. D6005 (USA). The procedure was done regarding the manufacturer's instructions.

- **DNA amplification:**

Four primers were used to carry out a multiplex PCR protocol, to detect both *erm*A and *erm*C . The primers were designed according to [12]. The sequence of the first pair targeting erm A gene was: 5′GTTCAAGAAC AATCAATACA GAG3′ and 5′GGATCAGGAA AAGGACATTT TAC3′ amplifying a 421 bp DNA fragment. The sequence of the second pair targeting ermC: 5′GCTAATATTG TTTAAATCGT CAATTCC3′ and 5′GGATCAGGAA AAGGACATTT TAC3′ amplifying a 572 bp DNA fragment.

PCR was performed in 50 µl volume reaction mixtures containing 1µl of each primer, 5 µl of crude template DNA, Water, nudease-free16 µl and 25 µl DreamTAq Green PCR Master Mix. The Denaturation, the annealing and the Extension temperature were 95℃, 55℃ and 72℃ respectively for *erm*A and *erm*C. The number of cycles for them was 25-40 cycles. Final extension done on 72℃ for 5-15 min for one cycle. The products of PCR were separated by gel electrophoresis on 1.5% agarose gel containing 2.5µg/ml ethidium bromide

RESULTS

The study was performed on 25 *S. aureus* isolates (Erythromycin intermediate and resistant. Clindamycin susceptible) by both Disc diffusion method and Vitek-2

system. MRSA was revealed in 18 (72%) of S. aureus isolates by Cefoxtin screening by both methods.

ICR was detected in 13 (52%) and 11 (44%) of S. aureus isolates by D test (Fig: 1) and Vitek 2 system respectively. Automated Vitek 2 system was unable to detect 2 positive ICR isolates done by the D test. PPV and NPV of the Vitek 2 system was 100% and 84.6% respectively. The sensitivity and specificity of the test were 85.7% and 100% respectevily. (table 1)

All ICR isolates were MRSA by both disc diffusion method and Vitek 2 system.

About distribution of ICR *S. aureus* in different clinical samples no statistical significant differences were detected regards ICR in distinct clinical samples by D test and Vitek-2 system. (P = 1, 0.48) respectively. (table 2)

By multiplex PCR showed that *erm* C and A genes were found in (40%) and (24%) of the studied isolates respectively. Both C and A genes were detected in (12%). (table 3, Fig: 2)

Table 1: Prevalence of ICR among S. aureus by D test and Vitek 2 system:

	D-test +ve (no.=13/25=52.0%)	**D-test –ve** (no.=12/25=48.0%)
Vitek-2 test +ve (11/25= 44.0%)	11	0
Vitek-2 test -ve (14/25= 56.0%)	2	12
Sensitivity (%)	85.7	
Specificity (%)	100.0	
PPV (%)	100.0	
NPV (%)	84.6	

*PPV: positive predictive value
*NPV: negative predictive value

Table 2: Distribution of ICR S. aureus in different clinical samples:

All=25	**Sputum (no.=9)**		**BAL (no.=3)**		**Abscess (no.=8)**		**Burn (no.=5)**		**P***
	No.		**No.**		**No.**		**No.**		
D + test (13)	5	55.56	1	33.33	4	50.0	3	60.0	1.00
Vitek 2 (11)	5	55.56	0	0.0	4	50.0	2	40.0	0.48

*Fisher Exact Test

Table 3: Distribution of erm A gene and erm C gene among the studied isolates:

No.=25	***erm* A only**		***erm* C only**		***erm* A+C**		**No *erm* A+C**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
No of strains	6	24.0	10	40.0	3	12.0	6	24.0

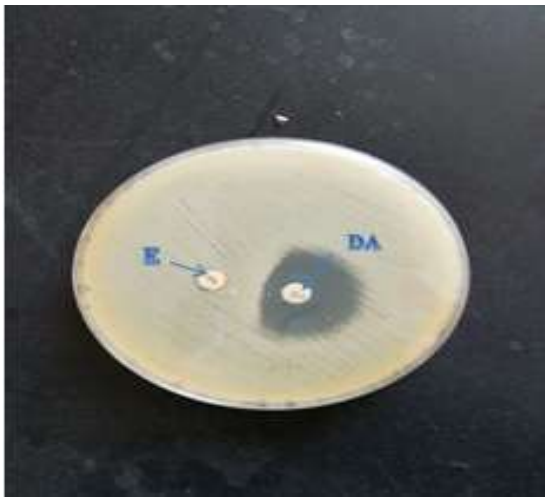

Fig: 1: D test positive.

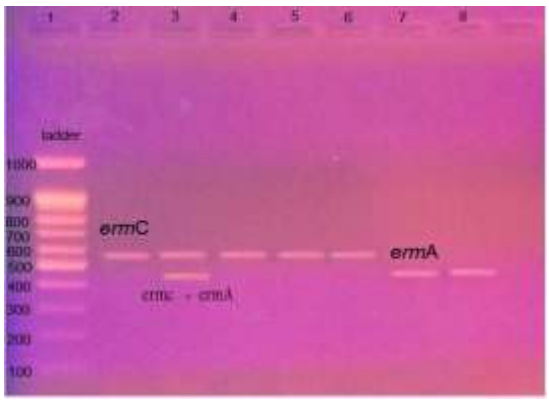

Fig 2: The results of multiplex PCR by gel electrophoresis

Lane 1: DNA Ladder 100 bp , Lane 2,4,5 and 6: *erm* C positive (572 bp), Lane 7 and 8: *erm* A positive (421 bp), Lane 3: *erm* C+ *erm* A.

DISCUSSION

Clindamycin, an antimicrobial belonging to the MLSB family, is frequently used for the treatment of skin and soft tissue infections because of *S. aureus*. However, the widespread use of the MLSB family of antimicrobials arrived to the emergence of resistance. Isolates with ICR are found to be resistant to erythromycin but susceptible to clindamycin when these discs are not put adjacent to each other during antimicrobial sensitivity testing. [13].

Conversely, labeling all EryR staphylococci as ClinR or not will likely prevent the use of Clin in treating infections that would likely respond to Clin therapy [14].

A series of the VITEK systems (bioMerieux, Marcy l'Etoile, France) has found to be a fully automated instrument that helps in species identification and antimicrobial susceptibility testing for a different clinical isolates, and are now used in many clinical microbiology laboratories worldwide. [15; 16].

The VITEK Advanced Expert System (AES) has been created to analyze the AST results using the well-established knowledge base of approximately 100 species and 20000 ranges of MIC to reveal more than 2300 phenotypic antimicrobial resistances. [17]

In the present study, by comparing the automated Vitek-2 system for detection of ICR with the results of the D-test as a gold standard technique. The sensitivity of the Vitek-2 test was 85.7% and the specificity was 100%. The PPV and NPV were 100% and 84.6% respectively. The automated system failed to detect 2 isolates as ICR positive while confirmed by D test.

Similar sensitivities were reported by Lavallée et al.[9], Nimmo et al.[19], Jethwani et al. [20], Buchan et al.[18], Gardiner et al.[2], reported a 93%,92.5%, 95.4% , 91.1%, 95% respectively.

The specificity and PPV of Vitek-2 test in this study were (100%), these findings were confirmed by many studiess. Nakasone et al.[17], Lavallée et al.[9] and Jethwani et al. [20] who reported the same results of us as specificity of the test with no false positive results. They recommended that positive vitek 2 results should be reported without confirmation by D test.

A probable reason for the false negatives of the ICR test is deficient in incubation time in the Vitek-2 for induction to occur. The card is incubated for 4–10 hours, with different time being relied on the inoculum and organism growth characteristics. If a slower growing organism is inoculated at the lower end of the recommended range of 0.5–0.63 MacFarlane, incomplete incubation time may contribute to a false negative result. [2]

The predominance of ICR among MRSA, MSSA in this study illustrated that all ICR positive were MRSA. This results coincided with Renushri, et al.[13] who reported that more MRSA isolates showed higher MLSBi phenotype (27.8%) compared to MSSA isolates (5.8%).

In contrast to our results Buchan et al. [18] showed that from 43 ICR positive S. aureus isolates MRSA was 15 isolates while MSSA was 28 isolates.

In this study frequency of ICR in clinical samples were 60%, 55.56%, 50% and 33% from burn, sputum, abscess and BAL respectively. The results showed a high similarity with Taie[21] who reported the most frequent positive (D Test) from Blood samples 4(4.4%), followed by Pus samples 2(2.2%), but the least frequent were from sputum samples 1(1.1%).

According to the phenotype and genotype association for the studied S. aureus isolates , the occurance of erm C gene and erm A gene were detected in (40%) and (24%) of the studied isolates respectively.

Both C and A genes were detected in (12%). None C and A genes were detect in 24%.

Our findings agreed with Pereira et al.[22] who reported that in the 44 isolates subjected to PCR, the *erm* C gene was detected in higher frequency than the *erm* A gene17 (38.6%) and 4 (9.1%) respectively. The occurrence of both gene was detected in only one (2.3%) of the isolates.

However Hosseini et al.[23] found that 10 MRSA isolates were inducible phenotype among which 5 isolates harbored *erm* A and 3 other isolates only contained *erm* C gene.

A possible explanation for the predominance of *erm* C positive MRSA isolates in this study was cleared by Spiliopoulou et al.[24] who reported that their isolates were belonged to two different MRSA clones although they were isolated in different hospitals. The predominance of *erm* C positive isolates was likely due to the selection and spread of these two multi-resistant MRSA clones.

CONCLUSIONS

The study highlights the crucial role of antibiotic susceptibility testing. As clindamycin is one of the most commonly used antibiotics for MRSA isolates. High prevalence of ICR among MRSA isolates decreases the therapeutic options for MRSA to the antibiotics as linezolid and vancomycin. The D test is an uncomplicated, reliable and not expensive test to perform along with routine susceptibility testing which detects the iMLSB and the cMLSB resistance. Vitek-2 system is a reliable method for antimicrobial testing including ICR. Vitek-2 system combined with AES will extremely contribute to laboratory function in our field of clinical microbiology. The predominance of *erm* C as the genetic determinant that responsible for the expression of resistance to MLS antibiotics among the total S. aureus population. Also, increasing of (*erm*A + *erm*C) genes in the MRSA emphasizes on the accurate use of these antibiotics to stop any treatment failure.

REFERENCES

1. Mojtaba Moosavian, Saeed Shoja, Soodabeh Rostami, Maryam Torabipour and Zahra Farshadzadeh.): Inducible clindamycin resistance in clinical isolates of Staphylococcus aureus due to erm genes. Iranian Journal of Microbiology.2014.Vol. 6 No. 6 421-427.
2. Gardiner BJ, Grayson ML and Wood GM.: Inducible resistance to clindamycin in Staphylococcus aureus: validation of Vitek-2 against CLSI D-test. Pathology.2012. 45(2), pp. 181 – 184.
3. Bernard AL, Kermarrec G, Parize P, Caruba T, Bouvet A, Mainardi JL, Sabatier B, Nich C: Dramatic reduction of clindamycin serum concentration in staphylococcal osteoarticular infection patients treated with the oral clindamycin-rifampicin combination. Journal of Infection.2015. 71,:200-206.
4. Jarajreh D, Aqel A, Alzoubi H, Al-Zereini W: Prevalence of inducible clindamycin resistance in methicillin-resistant Staphylococcus aureus: the first study in Jordan. J Infect Dev Ctries. 2017.11(4):350-354.
5. Singh E, Manjunath V, GRT, Prakash MR, Sharma A: D-Test – Its role in detection of inducible resistance to Clindamycin in Staphylococcus aureus with special reference to MRSA. Int. J. Biol. Med. Res.2012; 3(1): 1430-1432.
6. Steward CD, Raney PM, Morrell AK, Williams PP, McDougal LK, Jevitt L, et al.: Testing for induction of clindamycin resistance in erythromycin-resistant isolates of Staphylococcus aureus. J Clin Microbiol;2005.43:1716-1721.
7. O'Sullivan MV, Cai Y, Kong F, Zeng X, Gilbert G.: Influence of Disk Separation Distance on Accuracy of the Disk Approximation Test for Detection of Inducible Clindamycin Resistance in Staphylococcus spp. Journal of Clinical Microbiology. 2006. 44: 4072–4076.
8. April M. Bobenchik, Janet A. Hindler, Carmen L. Giltner, Sandra Saeki,b Romney M. Humphriesa.): Performance of Vitek 2 for Antimicrobial Susceptibility Testing of staphylococcus spp. and Enterococcus spp. Journal of Clinical Microbiology. 2014 Vol. 52 No. 2. p. 392–397.
9. Christian Lavallée, Danielle Rouleau, Christiane Gaudreau, Michel Roger, Catherine Tsimiklis, Marie-Claude Locas, Simon Gagnon, Jocelyn Delorme and Annie-Claude Labbé.): Performance of an Agar Dilution Method and a Vitek 2 Card for Detection of Inducible Clindamycin Resistance in Staphylococcus spp. Journal of Clinical 1Microbiology, Apr. 2010, Vol. 48, No. 4, p. 1354–1357.
10. CLSI: Performance Standards for Antimicrobial Susceptibility Testing. This edition provides updated tables for the Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards M02-A12, M07-A10, and M11-A8. 26th Edition.2016.
11. VITEK® 2 Systems Product Information:510809-2EN1 -2422,2008.
12. Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J.: Distribution of genes encoding resistance to macrolides, lincosamides,

and streptogramins among Staphylococci. Antimicrob. Agents Chemother.1999, 43: 10621066.

13. Renushri, Saha A, Nagaraj, Krishnamurthy V: Inducible Clindamycin Resistance in Staphylococcus aureus Isolated from Nursing and Pharmacy Students. Journal of Laboratory Physicians: 2011,Vol-3 :Issue-2.
14. Fokas S, Tsironi M, Kalkani M, Dionysopouloy M: Prevalence of inducible clindamycin resistance in macrolide-resistant Staphylococcus spp. Clin. Microbiol. Infect.2005, 11:337–340.
15. Funke G, Funke-Kissling P: Performance of the new VITEK 2 GP card for identification of medically relevant Gram-positive cocci in a routine clinical laboratory. J Clin Microbiol.2005, 43:84 – 88.
16. Aubertine CL, Rivera M, Rohan SM, Larone DH: Comparative study of the new colorimetric VITEK 2 yeast identification card versus the older fluorometric card and of CHROMagar Candida as a source medium with the new card. J Clin Microbiol.2006, 44:227 – 228.
17. Nakasone I, Kinjo T, Yamane N, Kisanuki K, and Shiohira CM.: Laboratory-based evaluation of the colorimetric VITEK-2 Compact system for species identification and of the Advanced Expert System for detection of antimicrobial resistances: VITEK-2 Compact system identification and antimicrobial susceptibility testing. Diagn. Microbiol. Infect. Dis.2007, 58: 191-198.
18. Buchan BW, Anderson NW, Ledeboer NA.: Comparison of BD Phoenix and bioMerieux Vitek 2 automated systems for the detection of macrolide lincosamide-streptogramin B resistance among clinical isolates of Staphylococcus. Diagn Microbiol Infect Dis.2012, 72: 291–4.
19. Nimmo G, Pearson J, Collignon P, Christiansen K, Coombs G, Bell J, McLaws ML.: Staphylococcus aureus Survey 2011 Antimicrobial Susceptibility Report (SAP 2011). Perth: Australian Group on Antimicrobial Resistance (AGAR), www.agargroup.org.
20. Jethwani UN, Mulla SA, Shah LN, Panwala TR.: Detection of inducible clindamycin resistance by an automated system in a tertiary care hospital. Afr J Microbiol Res.2011, 5: 2870–2.
21. Taie DH: Inducible inducible clindamycin resistance among clinical isolates of Staphylococcus aureus.2013. M.Sc. Thesis in Clinical Pathology Department, Faculty of Medicine Menoufiya University.
22. Pereira JNP, Rabelo MA, Lima JLC, Neto AMB, Lopes ACS, Maciel MAV: Phenotypic and molecular characterization of resistance to macrolides, lincosamides and type B streptogramin of clinical isolates of Staphylococcus spp. of a university hospital in Recife, Pernambuco, Brazil. braz j infect dis.2016, 2 0(3) : 276–281.
23. Hosseini SS, Niakan M, Saderi H, Motallebi M, Taherikalani M, Asadollahi K, Emaneini M: Frequency of genes encoding erythromycin ribosomal methylases among Staphylococcus aureus clinical isolates with different D-phenotypes in Tehran, Iran. IRAN. J. MICROBIOL.2016, 8:3 .161-167.
24. Spiliopoulou I, Petinaki E, Papandreou P, Dimitracopoulos G.: erm(C) is the predominant genetic determinant for the expression of resistance to macrolides among methicillin-resistant Staphylococcus aureus clinical isolates in Greece. Journal of Antimicrobial Chemotherapy.2004, DOI: 10.1093/jac/dkh197.