Correlation between reduced susceptibility to disinfectants and multidrug resistance among clinical isolates of Acinetobacter species

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Background: The aim of this study was to investigate the susceptibility profiles to disinfectants and antimicrobial agents of 283 non-repetitive Acinetobacter clinical isolates obtained in 97 Japanese hospitals in March 2002.

Methods: Susceptibility profiles of the above isolates to four disinfectants, six antimicrobial agents and two dyes were investigated. MICs were measured by the agar dilution method recommended by the CLSI (formerly NCCLS). MBC measurements and time–kill assays were performed using a slightly modified quantitative suspension test based on the European Standard EN 1040.

Results: No evident resistance to disinfectants was seen among the 283 strains of Acinetobacter spp. isolated in 2002, but the MIC90s of chlorhexidine gluconate, benzalkonium chloride and alkyldiaminoethylglycine hydrochloride were 50, 50 and 400 mg/L, respectively. Interestingly, the MICs of alkyldiaminoethylglycine hydrochloride and benzethonium chloride for four and three clinical isolates, respectively, reached 800 mg/L (approximately half the in-use concentration). The MBCs for the 28 disinfectant reduced susceptibility (DRS) isolates, for which the MICs of at least one of the four disinfectants tested were higher than the MIC90, were comparable to those for susceptible isolates, in general; however, significant differences (P < 0.01) were observed between disinfectant-susceptible and DRS isolates in the time–kill assays of chlorhexidine gluconate, benzalkonium chloride and benzethonium chloride. Furthermore, DRS isolates tended to demonstrate multiresistance profiles to ceftazidime, ciprofloxacin and amikacin (P < 0.05).

Conclusions: Since several Acinetobacter clinical isolates have developed augmented resistance to multiple antimicrobials and disinfectants, it is worth checking the susceptibility to disinfectants if multidrug-resistant Acinetobacter spp. are recurrently isolated clinically.

Keywords: time-dependent survey, nosocomial pathogen, antimicrobial agent, infection control

Introduction

Acinetobacter spp., especially Acinetobacter baumannii, have emerged as a major cause of nosocomial infections, particularly in intensive care units (ICUs), where immunocompromised patients are routinely prescribed various antimicrobial agents.1–4 Furthermore, Acinetobacter spp. have an innate ability to readily accept foreign DNA, including genetic determinants for antimicrobial resistance, so as to adapt to and survive in environments that are hazardous to bacterial growth.5,6 Therefore, they have a propensity for developing resistance to multiple classes of antimicrobial agents, including broad-spectrum β-lactams, fluoroquinolones and aminoglycosides.7,8 Recently, even in military medical facilities, a series of infections, such as osteomyelitis and cutaneous infections, caused by multidrug-resistant A. baumannii was reported.7 The intrinsic abilities of this microbe to rapidly develop antimicrobial multidoesistance and to survive long-term on dry surfaces have also been considered to play a crucial role in hospital-acquired infections.8,9

Biocides, including antiseptics and disinfectants, have been used extensively in hospitals and other healthcare settings for the sterilization of various medical devices and surfaces of nosocomial environments. In particular, disinfectants play an essential role in infection control and the prevention of nosocomial transmission of infectious microorganisms.10 The benefits of the introduction of comprehensive disinfection on the reduction of healthcare-associated infections have been described,11 although reduced susceptibility to biocides has been described...
for various nosocomial pathogens, such as methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa.12,13 Unlike these pathogens, few studies have investigated the susceptibility to disinfectants in Acinetobacter spp.14,15 More recently, we reported that repeated exposure to subinhibitory concentrations of chlorhexidine gluconate gradually elevated its MIC for Acinetobacter spp., especially for those isolates demonstrating reduced susceptibility to disinfectants.16

Together with the development of multiple antimicrobial resistance in nosocomial pathogens, correlations between the levels of multiple antimicrobial resistance and MICs of biocides have been investigated.11,17 However, the available information about the linkage of resistance profiles to disinfectants and antimicrobial agents has so far been limited to a few bacterial species. Accordingly, presently existing data seem insufficient for the empirical application of disinfectants. Acinetobacter spp. have rapidly acquired resistance to multiple antimicrobial agents over the past 10 years, so greater understanding of the susceptibility to disinfectants among Acinetobacter spp. and information on the correlation between their susceptibility profiles to disinfectants and antimicrobials would contribute to the control of this microbe in both hospital and long-term healthcare settings.

Materials and methods

Bacterial strains

In March 2002, 283 non-repetitive clinical isolates identified as Acinetobacter spp. were collected from 97 hospitals located in different geographical areas of Japan. Since these isolates were speculated to be a probable causative microbe of infection in each patient, they were subjected to identification and antimicrobial susceptibility tests. They were biochemically identified as 273 A. calcoaceticus – baumannii complex, 7 Acinetobacter lwoffii and 3 Acinetobacter junii using the API20NE system (BioMérieux Japan, Tokyo, Japan) and a complementary test for their growth at 37, 41 and 44°C. 

Disinfectants, antimicrobial agents and susceptibility tests

Disinfectants and antimicrobial agents were obtained from the following sources: chlorhexidine gluconate, benzethonium chloride, gentamicin, amikacin, acriflavine, tetracycline and ethidium bromide, Wako Pure Chemical Industries, Osaka, Japan; benzalkonium chloride, Kanto Chemical, Tokyo, Japan; alkyldiaminoethylglycine hydrochloride [TEGO 51TM, Hokkaido Chemical Industries, Osaka, Japan]; benzalkonium chloride [511000, Alfresa Pharma, Osaka, Japan]; ceftazidime, GlaxoSmithKline, Tokyo, Japan; imipenem, Banyu Pharmaceutical, Tokyo, Japan; alkyldiaminoethylglycine hydrochloride [TEGO 51TM, Hokkaido Chemical Industries, Osaka, Japan]; benzalkonium chloride, Kanto Chemical, Tokyo, Japan; ciprofloxacin, Bayer Pharmaceutical, Leverkusen, Germany; amikacin, acriflavine, tetracycline and ethidium bromide, Wako Pure Chemical Industries, Osaka, Japan; benzalkonium chloride, Kanto Chemical, Tokyo, Japan; alkyldiaminoethylglycine hydrochloride [TEGO 51TM, Hokkaido Chemical Industries, Osaka, Japan]; benzalkonium chloride, Kanto Chemical, Tokyo, Japan; ciprofloxacin, Bayer Pharmaceutical, Leverkusen, Germany; amikacin, acriflavine, tetracycline and ethidium bromide, Wako Pure Chemical Industries, Osaka, Japan; benzalkonium chloride, Kanto Chemical, Tokyo, Japan; alkyldiaminoethylglycine hydrochloride [TEGO 51TM, Hokkaido Chemical Industries, Osaka, Japan]; benzalkonium chloride, Kanto Chemical, Tokyo, Japan; ciprofloxacin, Bayer Pharmaceutical, Leverkusen, Germany. MICs of disinfectants and antimicrobial agents were determined by the agar dilution method, according to the protocol recommended by the CLSI (formerly NCCLS) in document M100-S14.18 Appropriate dilutions of antimicrobial or disinfectant solutions were added to Mueller–Hinton agar (Becton Dickinson, Sparks, MD, USA) that had been allowed to equilibrate in a water bath to 50–55°C. The agar and antimicrobial or disinfectant solution were mixed thoroughly, and the mixture was poured into Petri dishes on a level surface to result in an agar depth of 3–4 mm. Each bacterial culture was adjusted to a turbidity equivalent to that of a 0.5 McFarland standard (~1–9×10^8 cfu/mL for most species) and was then diluted 1:10 in sterile Mueller–Hinton broth (Becton Dickinson, Sparks, MD, USA). A 5 μL aliquot of each diluted bacterial suspension containing ~10^8 cfu was spotted onto the agar surface using an inocula-replicating device [microplanter model MIT-P (Sakuma, Tokyo, Japan)] within 15 min of preparation and the plates were incubated at 35°C for 20 h. The MIC was recorded as the lowest concentration of the antimicrobial agent or disinfectant that completely inhibited growth, except for a single colony or a faint haze caused by the inoculum. A. calcoaceticus ATCC 33304 and 33305 were purchased from the ATCC, and served as the control strains in the antimicrobial susceptibility tests.

Assay of bactericidal activity: quantitative suspension test

The bactericidal effects of disinfectants on Acinetobacter spp. were measured using a slightly modified quantitative suspension test based on the protocol of European Standard EN 1040.19 Although a 5 min contact time is required in EN 1040, we changed the contact time to a more challenging 3 min to evaluate subtle differences between disinfectant-susceptible isolates and the disinfectant-resistant susceptible (DRS) isolates, as observed in the Results section. Each isolate was cultivated in Luria-Bertani (LB) broth (Becton Dickinson) until its optical density (OD) reached 0.90 at 660 nm. After washing once with PBS (pH 7.4), the bacterial test suspensions were adjusted to an OD of 0.08 at 660 nm (~10^8 cfu/mL). A 100 μL test suspension was added to 900 μL of disinfectant solutions and this reaction mixture was left for 3 min at 20±2°C. One hundred microlitres of the mixture was added to 900 μL of neutralizer solution [10% Tween 80, 3% lecithin, 0.1% histidine, 0.5% sodium thiosulphate and PBS (pH 7.4)], kept at 20±2°C for 3 min and then serially diluted in PBS. After dilution, 50 μL of the mixture was spread immediately onto LB agar plates and incubated for 18 h at 35°C. The number of colonies growing on each plate was counted and cell survival rates were calculated with those obtained by a test using a bacterial suspension treated with PBS instead of disinfectant as the control. The experiments were repeated three times on different days. In addition, the possible toxicity of the neutralizer towards the test organisms and the inactivation of the bactericidal activity of each disinfectant by the neutralizer were also assessed, as described previously.16

Measurement of MBCs and time–kill assays

MBC measurements and time–kill assays were performed to evaluate the bactericidal effects of four disinfectants using the quantitative suspension test described above. The MBC was determined using the disinfectant solutions at different concentrations made by serial 2-fold dilutions of each disinfectant. The MBC was defined as the lowest concentration that resulted in a 5 log_{10} reduction in the number of live bacterial cells for each disinfectant. Time–kill assays were performed at the lowest MIC of each disinfectant obtained by the agar dilution method, and exposure times to each disinfectant were 0, 10, 30, 60, 180, 300 and 600 s, respectively. Although dilution conditions employed for the MBC measurements and time–kill assays were usually selected in consideration of clinical practice, concentrations lower than the in-use concentrations of disinfectants were used in this study to evaluate the subtle differences between isolates with disinfectant-susceptible and reduced-susceptibility phenotypes. The experiments were repeated three times on different days.

Evaluation of bactericidal activity and statistical analyses

Data were analysed using the statistical program SPSS for Windows version 11.0J (SPSS Inc., Chicago, IL, USA). The correlation between MIC values of each disinfectant and the correlation between MICs of disinfectants and antimicrobial agents were determined using Spearman rank correlation. The Mann–Whitney U-test was performed to compare the bactericidal activities measured by both MBC and time–kill assays, as...
described above, and to compare MIC distributions between isolates with both disinfectant susceptibility and DRS. A value of $P<0.05$ was considered to be statistically significant.

**Results**

**Susceptibility to disinfectants**

Distributions of MICs of chlorhexidine gluconate, benzalkonium chloride, benzethonium chloride and alkyldiaminoethylglycine hydrochloride for 283 clinical isolates are shown in Table 1. *Acinetobacter* spp. tended to be susceptible to both chlorhexidine gluconate and benzalkonium chloride, and the MIC90s of these agents were 50 mg/L. However, the MICs of alkyldiaminoethylglycine hydrochloride were relatively higher than those of the other three disinfectants and the MIC90 of alkyldiaminoethylglycine hydrochloride was 400 mg/L. For several isolates, benzenthonium chloride and alkyldiaminoethylglycine hydrochloride showed a high MIC (800 mg/L), which was approximately half of the in-use concentration [0.2% (w/v)=2000 mg/L]. Table 1 shows a summary of the in-use concentration stipulated in the manuals for hand disinfection and the sterilization of various medical devices.

**Correlation between MIC values of disinfectants**

The possible correlation between the MIC values was examined. MICs of benzalkonium chloride for each isolate were correlated with those of benzenthonium chloride ($r=0.631$, $P<0.01$), because these two agents belong to the same class on the basis of chemical structure and mode of action. However, significant correlations were found between the MIC values of the other disinfectants for which the chemical structures and modes of action are dissimilar, e.g. benzalkonium chloride and chlorhexidine gluconate ($r=0.632$, $P<0.01$), benzethonium chloride and chlorhexidine gluconate ($r=0.425$, $P<0.01$), chlorhexidine gluconate and alkyldiaminoethylglycine hydrochloride ($r=0.643$, $P<0.01$), benzalkonium chloride and alkyldiaminoethylglycine hydrochloride ($r=0.657$, $P<0.01$) and benzethonium chloride and alkyldiaminoethylglycine hydrochloride ($r=0.520$, $P<0.01$).

**Selection of isolates with reduced susceptibility to disinfectants**

In the present study, the DRS isolates were defined as those for which the MICs of at least one among the four disinfectants were higher than the MIC90 when measured by the agar dilution method. As a result, 28 (9.9%) (19 A. baumannii, 5 A. calcoaceticus, 1 A. Iwoffii and 3 A. junii) of 283 isolates were provisionally defined as DRS isolates in the present study (Table 2). Most of the DRS isolates tended to demonstrate reduced susceptibility to two or more disinfectants.

**Bactericidal activity**

To evaluate the difference between isolates with disinfectant-susceptible and reduced-susceptibility phenotypes, 20 isolates for which MICs of all four disinfectants were lower than the MIC90 were defined as ‘disinfectant-susceptible isolates’ and randomly selected as the control strains for the MBC measurements and time–kill assays. Figure 1 shows that MBC values (mg/L) of chlorhexidine gluconate, benzalkonium chloride, benzethonium chloride and alkyldiaminoethylglycine hydrochloride for the disinfectant-susceptible isolates and the DRS isolates were 16 and 32, 12 and 32, 16 and 32, and 16 and 64, respectively, and 2- or 4-fold differences were observed for the MBCs of each disinfectant between disinfectant-susceptible isolates and DRS isolates. Moreover, a statistical significance ($P<0.05$) was found between the disinfectant-susceptible isolates and DRS isolates in the reduction of bacterial cells after exposure to chlorhexidine gluconate (8 and 16 mg/L), benzalkonium chloride (4 and 8 mg/L), benzethonium chloride (8 and 16 mg/L) and alkyldiaminoethylglycine hydrochloride (16 and 32 mg/L) (Figure 1).

As shown in Figure 2, bacterial cells of the disinfectant-susceptible isolates showed significant (5 log10) reductions after 180 s of exposure to all four disinfectants. On the other hand, the live bacterial cells of the DRS isolates gradually decreased after exposure to chlorhexidine gluconate, benzalkonium chloride and benzethonium chloride in comparison with the disinfectant-susceptible isolates, and the exposure times

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**Table 1. Distributions of MICs (mg/L) of various disinfectants by the agar dilution method**

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>In-use concentration (mg/L)$^a$</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>800</th>
<th>MIC50$^c$</th>
<th>MIC90$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHX</td>
<td>5000</td>
<td>0</td>
<td>0</td>
<td>175$^b$</td>
<td>28</td>
<td>67</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>B2K</td>
<td>2000</td>
<td>0</td>
<td>0</td>
<td>41</td>
<td>153</td>
<td>76</td>
<td>6</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>BZT</td>
<td>2000</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>12</td>
<td>122</td>
<td>113</td>
<td>7</td>
<td>16</td>
<td>3</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>ADH</td>
<td>2000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>160</td>
<td>72</td>
<td>40</td>
<td>4</td>
<td>100</td>
<td>400</td>
</tr>
</tbody>
</table>

CHX, chlorhexidine gluconate; B2K, benzalkonium chloride; BZT, benzethonium chloride; ADH, alkyldiaminoethylglycine hydrochloride.

$^a$In-use concentrations stipulated in the manuals are shown. These concentrations are generally used for hand hygiene and the disinfection of non-critical items, including medical devices, in hospital and other healthcare settings.

$^b$Bold numbers indicate groups of clinical isolates demonstrating the susceptible phenotype to each disinfectant. The 20 strains used for MBC measurements and time–kill assays were randomly selected from these groups.

$^c$Standard MIC50 and MIC90 measurements (quantal measurement of 50% and 90% of the population) are given. Twenty-eight isolates for which the MICs of at least CHX, B2K, BZT and ADH were above the MIC90 were defined as isolates with DRS and selected as candidates for MBC measurements and time–kill assays.
needed for their complete killing were prolonged to ~600 s in the DRS isolates. Significant differences (P<0.01) were also observed between disinfectant-susceptible isolates and DRS isolates in the time–kill assays of chlorhexidine gluconate (10 mg/L, at 30–300 s), benzalkonium chloride (10 mg/L, at 30–300 s) and benzethonium chloride (10 mg/L, at 60–300 s). The bactericidal activity of alkyldiaminoethylglycine hydrochloride on the DRS isolates was similar to that on the disinfectant-susceptible isolates and their bacterial cells showed a 5 log_{10} reduction after 180 s of exposure to disinfectants (Figure 2d).

**Susceptibility to antimicrobial agents and dyes**

For all 283 clinical isolates, the MICs of six antimicrobial agents and two dyes were determined. For the 28 clinical DRS isolates, MIC distributions apparently shifted towards resistance for all antimicrobials and dyes tested in comparison with those for disinfectant-susceptible isolates, suggesting acquisition of a higher level of resistance to these agents in the DRS isolates than in the disinfectant-susceptible isolates (P<0.05) (Figure 3). Moreover, susceptibilities of 283 isolates of Acinetobacter spp. to amikacin, ceftazidime, ciprofloxacin and imipenem were categorized into ‘susceptible’ (amikacin, ≤16 mg/L; ceftazidime, ≤8 mg/L; ciprofloxacin, ≤1 mg/L; and imipenem, ≤4 mg/L), ‘intermediate’ (amikacin, 32 mg/L; ceftazidime, 32 mg/L; ciprofloxacin, 4 mg/L; and imipenem, 8 mg/L) and ‘resistant’ (amikacin, ≥64 mg/L; ceftazidime, ≥32 mg/L; ciprofloxacin, ≥4 mg/L; and imipenem, ≥16 mg/L) in accordance with CLSI recommendations, and correlation coefficients between MICs of disinfectants and four antimicrobial agents
Issues in this species; however, there have been few studies of the antimicrobial resistance of the A. baumannii species. Many studies have focused on the antimicrobial resistance of A. baumannii strains belonging to EU clones I and II. The ability of specific strains to withstand in-use concentrations has been disseminated worldwide. Genetic studies have elucidated the potential of strains to disinfectants and antimicrobials. Bacterial strains belonging to Acinetobacter spp. are very common environmental microbes growing in soil, compost and drainage, but they have become one of the critical nosocomial pathogens due to the recent increase in their isolation from clinical samples. Extensive genotypic investigations on Acinetobacter spp. have confirmed that within A. baumannii, clusters of genetically very similar lineages have been disseminated worldwide. Of these, the European (EU) clones I, II and III have been widely spread across Europe as well as several countries outside of Europe, and the majority of the multidrug-resistant strains belong to EU clones I and II. The ability of specific clones such as EU clone II to acquire multidrug resistance would give them an advantage in antimicrobial-rich hospital environments and may have substantially facilitated their spread as nosocomial pathogens. Thus, the increasing frequency of infections caused by multidrug-resistant A. baumannii would accelerate the use of disinfectants as effective measures to prevent their inhabitation and transmission in healthcare settings. Many studies have focused on the antimicrobial resistance in this species; however, there have been few studies on the possible acquisition or development of reduced susceptibilities to disinfectants. Hence, the present study, focused on the correlations of susceptibilities of Acinetobacter spp. to antimicrobials and disinfectants isolated in 2002, is meaningful given the potential shift in the susceptibility profiles of Acinetobacter spp. to both antimicrobials and disinfectants.

In general, the term 'at-use concentration' has been used as the basis to define disinfectant-tolerant strains, though disinfectants are used for a variety of purposes. Thus, the 'at-use concentrations' vary considerably depending on the purposes of disinfection. In the present study, we used 'in-use concentrations', considering the hand hygiene of healthcare workers, and the disinfection of various non-critical medical devices used in clinical and healthcare settings. At present, the emergence of bactericidal-resistant clinical isolates, as was observed for Pseudomonas spp. that can withstand in-use concentrations of disinfectants, was not found for the Acinetobacter spp. isolated in 2002. More precisely, 283 non-repetitive clinically isolated Acinetobacter spp. were subjected to susceptibility tests for four disinfectants and the MIC90s of the disinfectants were found to be ≤400 mg/L, lower than their in-use concentrations. These results are consistent with previous reports by Martro et al. and Wisplinghoff et al., who found no apparent development of resistance to disinfectants among clinically isolated Acinetobacter spp. Indeed, no evident correlation was found, e.g. between the resistance profiles to antimicrobial agents and biocides for the nine Acinetobacter spp. that caused a sustained ICU outbreak in Spain. However, the correlation between the resistance levels to antimicrobial agents and disinfectants observed in the present study differed from previous observations. In the present study, the exposure times required...
respectively. Cell viabilities were determined by plating serially diluted cell suspensions on LB plates. Results are expressed as the log10 reduction caused by a strain of among clinically isolated resistance between multiple antimicrobials and disinfectants but our observations imply a trend towards overall cross-

regions worldwide.

Figure 2. Results of time–kill assays. Time–kill assays were performed for the 28 DRS isolates selected in the same manner as for the MBC assay. Bacterial cells were treated with each disinfectant at 20±2°C. Each bacterial test sample was removed at 10, 30, 60, 180, 300 and 600 s, respectively. Cell viabilities were determined by plating serially diluted cell suspensions on LB plates. Results are expressed as the log10 reduction in cell counts compared with that of the control sample treated with PBS. Error bars represent standard deviations of results from three experiments. (a) Chlorhexidine gluconate (CHX); (b) benzalkonium chloride (BZK); (c) benzethonium chloride (BZT); and (d) alkyldiaminoethylglycine hydrochloride (ADH).

for complete killing were significantly more prolonged in DRS isolates than in disinfectant-susceptible isolates, and the DRS isolates also demonstrated considerably higher resistance levels to ceftazidime, imipenem, ciprofloxacin and/or aminoglycosides. These findings may well suggest divergence in the mode of acquisition of reduced susceptibility to disinfectants and the development of multiple antimicrobial resistance in Acinetobacter spp. isolated on separate continents or in different regions worldwide.

Thus far, the positive linkage between bacterial resistance and the use of biocides has been suggested. Russell et al. revealed that chlorhexidine gluconate resistance in Pseudomonas stutzeri correlated with resistance to polymyxin B, gentamicin, erythromycin and ampicillin. Similar observations were found for other nosocomial pathogens, such as MRSA and P. aeruginosa. Our results showed no apparent correlations between specific disinfectants and antimicrobial agents, but our observations imply a trend towards overall cross-resistance between multiple antimicrobials and disinfectants among clinically isolated Acinetobacter spp. A hospital outbreak caused by a strain of Proteus mirabilis demonstrating resistance to several antimicrobial agents, including gentamicin as well as chlorhexidine gluconate, was reported. Thus, the increased isolation of Acinetobacter spp. that had acquired multiple resistance to antimicrobials would be a good indicator for early recognition of the emergence of Acinetobacter DRS isolates in both acute and long-term healthcare settings.

Figure 2. Results of time–kill assays. Time–kill assays were performed for the 28 DRS isolates selected in the same manner as for the MBC assay. Bacterial cells were treated with each disinfectant at 20±2°C. Each bacterial test sample was removed at 10, 30, 60, 180, 300 and 600 s, respectively. Cell viabilities were determined by plating serially diluted cell suspensions on LB plates. Results are expressed as the log10 reduction in cell counts compared with that of the control sample treated with PBS. Error bars represent standard deviations of results from three experiments. (a) Chlorhexidine gluconate (CHX); (b) benzalkonium chloride (BZK); (c) benzethonium chloride (BZT); and (d) alkyldiaminoethylglycine hydrochloride (ADH).

As combined resistance mechanisms to antimicrobials and disinfectants, augmented efflux pump functions and changes in permeability of the bacterial outer membrane have been reported. Although the antimicrobial resistance mechanisms of DRS isolates observed in the present study have not been well characterized, the recent rapid development of multiple antimicrobial resistance suggests the presence of potential common molecular mechanisms for augmented resistance levels to both disinfectants and antimicrobials. It seems difficult to conclude whether disinfectant exposure or antibiotic use is responsible for this phenomenon, but our data suggest the presence of a close genetic or mechanistic link between the antimicrobial resistance and DRS found among the clinical isolates.

In-use concentrations of disinfectants are much higher than their MICs and low-level resistance or DRS profiles have not been recognized as a possible clinical hazard to date. However, the clinical significance of emerging DRS Acinetobacter isolates deserves evaluation hereafter, given the close correlation with multiple antimicrobial resistance properties. Recent works report that carbapenem-resistant A. baumannii epidemic isolates belong to EU clonal complexes I or II and harbour blaoxa-23-like, blaoxa-40-like, blaoxa-60-like, blaoxa-51-like or blaoxa-58-like genes that have spread worldwide. Since the endemic A. baumannii strains isolated in Japanese clinical settings do not necessarily belong to the epidemic clonal complexes, susceptibility profiles to disinfectants among the global epidemic A. baumannii isolates belonging to the clonal complexes would be worth investigating, together
Figure 3. Comparison of MIC distributions between disinfectant-susceptible and DRS isolates. MICs of six antibiotics and two dyes for 283 clinical isolates of *Acinetobacter* spp. were compared between disinfectant-susceptible and DRS isolates. For the 28 clinical DRS isolates, MIC distributions apparently shifted to the right for all antimicrobials and dyes tested in comparison with those for disinfectant-susceptible isolates, suggesting acquisition of a higher level of resistance to these agents in the DRS isolates than in the disinfectant-susceptible isolates. White bars, MIC distributions for 255 disinfectant-susceptible isolates; black bars, MIC distributions for 28 DRS isolates. A significant difference between disinfectant-susceptible isolates and DRS isolates was indicated (*P* < 0.05; as determined by Mann–Whitney U-test). CAZ, ceftazidime; IPM, imipenem; GEN, gentamicin; AMK, amikacin; TET, tetracycline; CIP, ciprofloxacin; ACR, acriflavine; EtBr, ethidium bromide.
with their antimicrobial susceptibilities. Measures to prevent nosocomial infections caused by \textit{A. baumannii} should include appropriate usage of disinfectants. Indeed, the DRS isolates tend to be more tolerant of a group of biocides, but enforcement of correct disinfection regimens would still be essential when multidrug-resistant \textit{Acinetobacter} spp. are recurrently or continuously isolated in clinical settings despite enhanced performance of appropriate standard and contact precautions.

In conclusion, no apparent acquisition of resistance to disinfectants was observed in this time-dependent survey using the 283 strains of \textit{Acinetobacter} spp. clinically isolated in Japan in 2002. About 10\% of the isolates (28 strains) were found to demonstrate reduced susceptibility to disinfectants and these DRS isolates also tended to show resistances to various antimicrobial agents. Compared with the disinfectant-susceptible isolates using \textit{in vitro} stepwise exposure including MBC measurements and time–kill assays, the DRS isolates tend to survive much longer in sub-MIC concentrations of several disinfectants. Thus, susceptibility to disinfectants must be carefully checked on a case-by-case basis if several multidrug-resistant \textit{A. baumannii} are recurrently isolated from clinical specimens despite proper precautionary measures.

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**Table 3.** Correlation between MICs of disinfectants and antimicrobial agents

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Category (^a)</th>
<th>Number of strains</th>
<th>Median MIC (mg/L)</th>
<th>Spearman’s correlation coefficient (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CHX</td>
<td>BZK</td>
</tr>
<tr>
<td>CAZ</td>
<td>S</td>
<td>247</td>
<td>4</td>
<td>0.336 (P&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>13</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>23</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>IPM</td>
<td>S</td>
<td>268</td>
<td>0.5</td>
<td>0.095 (P=0.114)</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>13</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>CIP</td>
<td>S</td>
<td>252</td>
<td>0.25</td>
<td>0.224 (P&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>27</td>
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<td></td>
</tr>
<tr>
<td>AMK</td>
<td>S</td>
<td>268</td>
<td>2</td>
<td>0.189 (P&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>4</td>
<td>3</td>
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</tr>
<tr>
<td></td>
<td>R</td>
<td>11</td>
<td>64</td>
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</tr>
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</table>

CHX, chlorhexidine gluconate; BZK, benzalkonium chloride; BZT, benzethonium chloride; ADH, alkyl diaminoethylglycine hydrochloride; CAZ, ceftazidime; IPM, imipenem; CIP, ciprofloxacin; AMK, amikacin.

*\(^a\)Susceptibilities of 283 isolates of \textit{Acinetobacter} spp. to CAZ, IPM, CIP and AMK were categorized into susceptible (S), intermediate (I) and resistant (R) in accordance with CLSI criteria.*

**Transparency declarations**

None to declare.

**Supplementary data**

Figure S1 is available as Supplementary data at \textit{JAC} Online (http://jac.oxfordjournals.org/).

**References**

Susceptibility of *Acinetobacter* spp. to disinfectants and antimicrobials


