Reduced susceptibility to chlorhexidine disinfectant among New Delhi metallo-beta-lactamase-1 positive Enterobacteriaceae and other multidrug-resistant organisms: Report from a tertiary care hospital in Karachi, Pakistan

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Abstract

We analysed susceptibility of multidrug-resistant organisms (MDROs) including New Delhi metallo-beta-lactamase-1 positive Enterobacteriaceae to chlorhexidine and compared results to their susceptible counterparts. Susceptibilities of chlorhexidine digluconate in a standard (CHX-S) preparation and two commercial disinfectants containing different CHX concentrations (2% w/v and 4% w/w) were performed. MDROs had narrower range of higher CHX-S minimum inhibitory concentrations (MICs) as compared to pan-sensitive organisms. The MIC values for commercial disinfectants products for MDROs were many folds higher (20–600 times), than CHX-S for in vitro use. Increasing antibiotic resistance among bacterial isolates can be an indirect marker of reduced susceptibility to chlorhexidine in hospital setting.

Key words: Chlorhexidine digluconate, minimum inhibitory concentration, multidrug-resistant organism

Introduction

With the emergence of multidrug-resistant organisms (MDROs), there is increasing concern about the possible association of resistance to commonly used disinfectants.[1] Pathogens have the capability to survive on dry surfaces for several days; hence, they are continuous source of transmission if effective infection control strategies are not utilised.[2] Chlorhexidine has a broad spectrum of antimicrobial activity and is used extensively in hospitals for various applications.[3] Despite its widely accepted use and efficacy, studies investigating the emergence of chlorhexidine resistance among micro-organisms are limited.[4] In particular, impact of New Delhi metallo-beta-lactamase-1 (NDM-1) gene in Gram-negative organisms susceptibility to it remains largely unknown.

In this study, we performed a comparative analysis of chlorhexidine susceptibility of MDROs including Enterobacteriaceae, methicillin-resistant Staphylococcus aureus (MRSA), vancomycin resistant Enterococcus (VRE) and Acinetobacter spp. (AC), and corresponding susceptible strains.

Methods

Chlorhexidine susceptibility testing was performed on two sets of organisms: (a) Pan-sensitive (PAN) group (124) that included methicillin-sensitive S. aureus (MSSA = 45), vancomycin sensitive Enterococcus (VSE = 14), PAN-AC (2), PAN-Escherichia coli (PAN-EC = 37) and PAN-Klebsiella pneumoniae (PAN-KP = 26); (b) MDRO (161), NDM-1 positive KP (37) and NDM-1 positive EC (25), MRSA (50), VRE (18), multidrug-resistant AC (28), and extremely drug-resistant AC (XDR-AC = 3).

Antimicrobial susceptibility testing and minimum inhibitory concentration (MIC) were performed using Vitek2 (bioMerieux France) and interpreted according to the Clinical and Laboratory Standards Institute breakpoints.[5]
Standard chlorhexidine digluconate susceptibility

The MICs of chlorhexidine digluconate susceptibility (CHX-S) (Sigma, St. Louis, MO, USA) were determined using agar dilution, with a concentration range of 0.25–256 μg/mL. KP ATCC 13883 and EC ATCC 25922 (chlorhexidine MIC 16 and 2 μg/mL, respectively) were included as controls.[6]

Clinical in-use chlorhexidine digluconate preparation susceptibility testing

The MICs of in use CHX (CHX-IU) preparations were determined using broth dilution method containing a series of 2-fold dilutions, ranging in concentration from 19.5 to 10,000 μg/mL.

Statistical analysis

The 90th percentiles of MICs (MIC 90) of various drugs for resistant organism groups, namely, MRSA, MDR-AC and NDM-positive Enterobacteriaceae, were calculated. Frequencies of isolates with different CHX MICs were determined. The mean difference in MICs of CHX-S between different groups of organisms and their 95% confidence intervals were computed and a Student’s t-test was run to determine the statistical significance of this difference.

Results and Discussion

A total of 285 clinical bacterial isolates from different clinical sources was selected for this study.

Standard chlorhexidine digluconate susceptibility

We found a narrower range of higher chlorhexidine MIC values for all MDROs as compared to pan-sensitive organisms as shown in Table 1. The current study showed Gram-negative bacteria to be more resistant to CHX-S (MIC range: 1–256 μg/ml) than Gram-positive bacteria (MIC range 0.5–32 μg/ml), as reported in previous studies.[1,7,8] Among Gram-positive bacteria VRE and Gram-negative bacteria colistin-resistant AC exhibited the highest chlorhexidine MICs as compared to other MDROs [Table 1]. This finding suggests that frequent use of colistin induces chlorhexidine resistance among Gram-negative bacteria. Both colistin and chlorhexidine act through outer cell membrane disruption with leakage of intracellular contents and bacterial death.[7] The mean difference in chlorhexidine MICs for MRSA versus MSSA, VRE versus VSE and NDM-1 positive-KP and NDM-1 positive-EC versus PAN-EC and PAN-KP was statistically significant (P < 0.0001) with a 95% confidence interval. Although the mean difference in chlorhexidine MIC values for MDR-AC versus PAN-AC was statistically significant (P = 0.013), the number of isolates was too few (n = 2). The MIC 90 values for all tested isolates are shown in Table 1.

In-use chlorhexidine digluconate preparation susceptibility

The MICs of CHX-IU1 and CHX-IU2 for MDROs were many times higher than CHX-S. For NDM-1 positive-EC, NDM-1 positive-KP, XDR-AC, MRSA, MSSA and VRE, the MIC values of CHX-IU1 were 20–150-fold higher and MIC values of CHX-IU2 were 40,600-fold higher than those of CHX-S. Both CHX-IU were found to have inhibitory effects at the maximum concentration (undiluted form) only and failed to inhibit bacterial growth when diluted. In most hospitals throughout Pakistan, activities related to housekeeping services including choice of disinfectants, preparations of disinfectants and frequency of preparations (daily versus weekly) are done by personnel with little or no formal education. Label instructions are often ignored, and with limited resources, the tendency may be to dilute the disinfectant for longer use. EC ATCC 25922 and KP ATCC 13883 exhibited highest chlorhexidine MIC >10000 μg/ml when tested against the two commercial preparations. These results suggest that the effective concentration of chlorhexidine in these products was 2500–5000-fold less than CHX-S. This finding raises a concern about the quality of the product and preparation on site which is alarming and reflects poor quality control in commercial product manufacturing in the local market. Alternatively, the breakdown of chlorhexidine due to storage at improper temperatures for extended periods of time might be responsible for high MIC values observed with commercial products. Use of sub-standard (diluted) disinfectants in hospital environmental cleaning gives ineffective infection control practices. Strict implementation of hospital disinfectant policies and legislative regulations in production and import of disinfectants are required.

Conclusion

In the absence of standardised methods for CHX-S, hospital antibiogram can be a useful predictor for CHX susceptibility among pathogens. Moreover, use of colistin might induce resistance to CHX in Gram-negative rods. Further studies are recommended to investigate this association. Proper implementations of hospital disinfectant policies coupled with strict manufacture regulations are strongly recommended.

Acknowledgements

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Nil.

Conflicts of interest

There are no conflicts of interest.
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<th>4 (µg/mL)</th>
<th>8 (µg/mL)</th>
<th>16 (µg/mL)</th>
<th>32 (µg/mL)</th>
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Pan-sensitive E. coli (25)
Pan-sensitive E. coli (37)
MRSA (50)
MSSA (45)
Vancomycin resistant Enterococcus (18)
Vancomycin sensitive Enterococcus (14)
MDR Acinetobacter spp. (28)
XDR Acinetobacter spp. (3)
Pan-sensitive Acinetobacter spp. (2)

References