A-087

Differential Potential for the Disinfectant Benzalkonium Chloride (BZK) and the Antiseptics Chlorhexidine (CHX) and Omiganan Pentahydrochloride to Serve as Resistance Selectors in the Hospital Environment

TR FRITSCHE, DM JOHNSON, HS SADER, RN JONES
JMI Laboratories, North Liberty, IA, USA

ASM 2007
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

ABSTRACT

Background: Continuous environmental exposure of pathogens to BZK (quaternary disinfectant) and CHX (skin antiseptic replacing iodaphors) in the hospital is a recognized risk factor for resistance emergence through selection for *qac*-containing class 1 integrons in Gram-negative bacilli and plasmids with *qac* or *smr* genes in *S. aureus* (SA). Development of a novel cationic peptide, omiganan, as an alternative skin antiseptic for the prevention of catheter-site infections, may prove beneficial in limiting R emergence. We summarize results comparing BZK, CHX and omiganan MIC and MBC values during a serial passaging experiment.

Methods: Tested strains including 4 SA (2 oxacillin-R [MRSA]) and 4 PSA (2 carbapenem [CARB]-R via VIM-1 and IMP-13) were serially passaged for 7 days using growth one $\log_2$ dilution below the MIC for subsequent inocula. MBC values were assessed by plating all wells ≥ MIC and assessing for ≥99.9% inoculum reduction. All isolates were susceptibility (S) tested using CLSI methods.

Results: While evidence of adaptive R or tolerance did not appear (see Table), BZK MIC and MBC values were markedly increased (8-fold) in both integron-containing (CARB-R) PSA over those of wildtype strains. Four-fold MIC and MBC increases following passaging were noted with CHX, occurring in wildtype SA and PSA strains. Omiganan showed 4-fold MBC increases following passaging in two PSA but no MIC increased over 2-fold.

	MIC/MBC (µg/ml)					
	BZK		CHX		Omiganan	
Organism	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
S. aureus						
MSSA, QC strain	2/4	2/4	1/2	2/2	16/16	32/64
MSSA, Wildtype 1	2/4	2/8	1/2	2/4	16/16	8/16
MRSA, Wildtype 2	8/8	8/8	1/1	4/4	16/32	16/32
MRSA, Wildtype 3	2/8	4/8	2/4	4/4	32/64	32/64
P. aeruginosa						
CARB-S, QC	64/128	64/128	4/8	8/16	128/128	256/512
CARB-S, Wildtype	64/64	64/64	4/8	16/32	64/128	64/128
CARB-R, VIM-1	512/512	512/512	8/8	16/16	128/256	256/512
CARB-R, IMP-13	512/512	512/512	8/32	16/32	256/256	512/1024

Conclusions: Widespread use of disinfectants and antiseptics in the hospital environment poses risks for resistance emergence. As shown here BZK MIC and MBC values were markedly increased (8-fold) in PSA with *qac*-containing class 1 integrons compared with wildtype strains. Likewise, both MIC and MBC values for CHX were increased 4-fold following passaging with wildtype SA and PSA strains. Use of an agent such as omiganan limited to patient topical applications at the clinical formulation concentration (1%; 10,000 µg/ml) may offer benefits of a broad-spectrum (bacteria and yeast) skin antiseptic but without concern for environmental R selection recognized with commonly used biocides.

INTRODUCTION

Given the widespread hospital use of quaternary ammonium compound (QAC) disinfectants and chlorhexidine (skin antisepsis replacing iodaphors), selection for class 1 integrons in Gram-negative bacilli and plasmids with *qac* or *smr* genes in *Staphylococcus aureus* could inadvertantly enrich the hospital environment with multidrug-resistant organisms. Selection for these resistance integrons and plasmids can occur in environments contaminated with disinfectants and perhaps, antiseptics, but conversely, can also be selected for by antimicrobial agents (ß-lactams, aminoglycosides, sulfonamides, etc). Recent publications suggest that increased resistance to fluoroquinolones, broad-spectrum ß-lactams and aminoglycosides, as observed in the routine antibiogram, can predict susceptibility to chlorhexidine. Other investigations have documented that class 1 integron incidence was significantly higher among bacterial populations that were pre-exposed to QACs, and that selection for QAC resistance may cause co-selection for antimicrobial resistance.

As a result, concern has been voiced over the potential for producing a reservoir of resistance in hospital environments following continuous low-level exposure to such chemical agents, and the threat this creates for the control of nosocomial infections. Utilization of agents that lack resistance selection (including cross-resistance) and emergence potential, is a critical approach that is needed to diminish the burden of such hospital-acquired infections.

We present results for a pilot evaluation to characterize the potential for key clinical pathogens to develop resistance to a disinfectant (benzalkonium chloride), an antiseptic (chlorhexidine) and omiganan pentahydrochloride following sublethal exposure; and secondly to determine bactericidal activity of these compounds prior to, and following, exposure to the respective agents. Omiganan is a novel cationic peptide being developed as a topical agent for prevention of catheter-site infections.

MATERIALS AND METHODS

Organisms studied: Tested species included methicillin-susceptible (107-1150D and ATCC 29213) and methicillin-resistant *S. aureus* (81-1476A and 2-1108A); and wild-type (66-728C and ATCC 27853) and metallo-ß-lactamase-producing *P. aeruginosa* (86-10088A [VIM-1 positive] and 86-14571A [IMP-13 positive]).

Susceptibility test methodologies: Tested agents included benzalkonium chloride (Sigma Chemical; testing range, 2048 to 1 µg/ml), chlorhexidine dihydrochloride (Sigma Chemical; testing range, 512 to 0.25 µg/ml) and omiganan pentahydrochloide (Cadence Pharmaceuticals; testing range, 2048 to 1 µg/ml). MIC values for these agents were determined using reference broth microdilution methods as specified in CLSI document M7-A7 (2006). Inoculum preparations, assay performance and quality control (QC) guidelines were those specified in CLSI M7-A7 (2006), M100-S17 (2007) and for omiganan, Anderegg et al (2004).

Selection of resistance: Organisms were serially passaged for seven days by selecting wells one dilution below the MIC (last well with growth) and using this growth to prepare the inoculum for subsequent testing; MIC values were recorded daily.

MBC values were assessed for benzalkonium chloride, chlorhexidine dihydrochloride and omiganan at the beginning and the end of the experiment by plating the broth from the MIC well and from those $\log_2$ dilutions above the MIC for each organism onto appropriate drug-free growth media. Quantitative colony counts were subsequently performed on the starting inoculum at the time the MIC test was performed. The lowest concentration of antimicrobial agent that killed ≥99.9% of the starting test inoculum was defined as the MBC endpoint (NCCLS M21-A and M26-A).

RESULTS

- Results of daily quality control for omiganan tested with *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853 were within limits as specified by Anderegg et al (2004).
- Both MRSA strains had baseline MIC values for chlorhexidine and omiganan that were equal to, or two-fold higher than those of the susceptible (MSSA) strains (Table 1).
- One MRSA benzalkonium MIC value was four-fold higher than for methicillin-susceptible strains. Chlorhexidine MIC values for all four strains increased two- to four-fold during passaging whereas only one strain each displayed a two-fold MIC increase for benzalkonium and omiganan.
- Also, two, one and one *S. aureus* strains showed MBC value increases for chlorhexidine (two- and four-fold), benzalkonium (two-fold) and omiganan (four-fold), respectively.
- Benzalkonium chloride MIC values for the carbapenem-resistant *P. aeruginosa* were markedly higher (eight-fold) than for susceptible strains; two-fold higher for chlorhexidine; and two- to four-fold higher for omiganan.
- MIC and MBC values for chlorhexidine increased for all *P. aeruginosa* strains during passaging (two- to four-fold). MIC and MBC values for omiganan also increased during passaging in three of four strains (two-fold and two-to four-fold, respectively).

Table 1. Determination of MIC and MBC values for *S. aureus* and *P. aeruginosa* following exposure to either benzalkonium chloride, chlorhexidine dihydrochloride or omiganan pentahydrochloride in a seven-day passaging experiment.

	MIC/MBC (µg/ml)					
	Benzalkonium-Cl		Chlorhexidine		Omiganan	
Organism[a]	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
S. aureus						
MSSA, QC strain	2/4	2/4	1/2	2/2	16/16	32/64
MSSA, Wildtype 1	2/4	2/8	1/2	2/4	16/16	8/16
MRSA, Wildtype 2	8/8	8/8	1/1	4/4	16/32	16/32
MRSA, Wildtype 3	2/8	4/8	2/4	4/4	32/64	32/64
P. aeruginosa						
CARB-S, QC	64/128	64/128	4/8	8/16	128/128	256/512
CARB-S, Wildtype 1	64/64	64/64	4/8	16/32	64/128	64/128
CARB-R, VIM-1	512/512	512/512	8/8	16/16	128/256	256/512
CARB-R, IMP-13	512/512	512/512	8/32	16/32	256/256	512/1024

a. MSSA = methicillin-susceptible *S. aureus*; MRSA = methicillin-resistant *S. aureus*; CARB-S = carbapenem-susceptible; CARB-R = carbapenem resistant.

CONCLUSIONS

- While evidence of adaptive resistance or tolerance did not appear in *P. aeruginosa*, benzalkonium chloride MIC and MBC values were markedly increased (eight-fold) in both strains exhibiting *qac*-containing class 1 integrons (carbapenem-resistant) over those of wildtype strains.
- Likewise, both MIC and MBC values for chlorhexidine were increased two- to four-fold following passaging of wildtype *S. aureus* and *P. aeruginosa* strains.
- Omiganan showed four-fold MBC increases following passaging in two *P. aeruginosa* strains but no MIC increased more than two-fold.
- Use of an agent such as omiganan limited to patient topical applications at the clinical formulation concentration (1%; 10,000 µg/ml) may offer benefits of a broad-spectrum (bacteria and yeast) skin antiseptic with the added benefit of not being a concern for environmental resistance selection often recognized with commonly used biocides.

SELECTED REFERENCES

1. Anderegg TR, Fritsche TR, Jones RN (2004). Quality control guidelines for MIC susceptibility testing of omiganan pentahydrochloride (MBI 226), a novel antimicrobial peptide. *J Clin Microbiol* 42: 1386-1387.
2. Bjorland J, Steinum T, Kvitle B, Waage S, Sunde M, Heir E (2005). Widespread distribution of disinfectant resistance genes among staphylococci of bovine and caprine origin in Norway. *J Clin Microbiol* 43: 4363-4368.
3. Block C, Furman M (2002). Association between intensity of chlorhexidine use and micro-organisms of reduced susceptibility in a hospital environment. *J Hosp Infect* 51: 201-206.
4. Braoudaki M, Hilton AC (2004). Adaptive resistance to biocides in *Salmonella enterica* and *Escherichia coli* O157 and cross-resistance to antimicrobial agents. *J Clin Microbiol* 42: 73-78.
5. Clinical and Laboratory Standards Institute. (2006). *M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - seventh edition*. Wayne, PA: CLSI.
6. Clinical and Laboratory Standards Institute. (2007). *M100-S17, Performance standards for antimicrobial susceptibility testing, 17th informational supplement*. Wayne, PA: CLSI.
7. Gaze WH, Abdouslam N, Hawkey PM, Wellington EM (2005). Incidence of class 1 integrons in a quaternary ammonium compound-polluted environment. *Antimicrob Agents Chemother* 49: 1802-1807.
8. Heir E, Sundheim G, Holck AL (1999). Identification and characterization of quaternary ammonium compound resistant staphylococci from the food industry. *Int J Food Microbiol* 48: 211-219.
9. Koljalg S, Naaber P, Mikelsaar M (2002). Antibiotic resistance as an indicator of bacterial chlorhexidine susceptibility. *J Hosp Infect* 51: 106-113.
10. McBain AJ, Ledder RG, Moore LE, Catrenich CE, Gilbert P (2004). Effects of quaternary-ammonium-based formulations on bacterial community dynamics and antimicrobial susceptibility. *Appl Environ Microbiol* 70: 3449-3456.
11. Noguchi N, Suwa J, Narui K, Sasatsu M, Ito T, Hiramatsu K, Song JH (2005). Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes qacA/B and smr of methicillin-resistant *Staphylococcus aureus* isolated in Asia during 1998 and 1999. *J Med Microbiol* 54: 557-565.
12. Russell AD (2004). Bacterial adaptation and resistance to antiseptics, disinfectants and preservatives is not a new phenomenon. *J Hosp Infect* 57: 97-104.
13. Sader HS, Fedler KA, Rennie RP, Stevens S, Jones RN (2004). Omiganan pentahydrochloride (MBI 226), a topical 12-amino-acid cationic peptide: Spectrum of antimicrobial activity and measurements of bactericidal activity. *Antimicrob Agents Chemother* 48: 3112-3118.
14. Sidhu MS, Heir E, Leegaard T, Wiger K, Holck A (2002). Frequency of disinfectant resistance genes and genetic linkage with beta-lactamase transposon TN552 among clinical staphylococci. *Antimicrob Agents Chemother* 46: 2797-2803.
15. Thomas L, Russell AD, Maillard JY (2005). Antimicrobial activity of chlorhexidine diacetate and benzalkonium chloride against *Pseudomonas aeruginosa* and its response to biocide residues. *J Appl Microbiol* 98: 533-543.