The impact of disinfectants on antimicrobial resistance
An Ayliffe prediction

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Disclosures

Copper Development Association Africa: research funds for Masters project:
  – “Viability of TB after re-aerosolization and the effect of copper”
  – “Water containers for household in endemic cholera areas”

The Impact of Disinfectants on Antimicrobial Resistance
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Prof G Ayliffe - Predictions

..”Aseptic methods are often poor, the use of disinfectants was excessive and inadequately controlled and defects in sterilization were common (Forward: Ayliffe’s Control of Healthcare Associated Infection, 2009)

..”however alternatives are sometimes necessary due to the lack of resources such as clean water, electricity & disposables” (Forward: Hospital Infection Control, Setting up a cost effective programme, 1992)

Global Market 2015

Global Antiseptic and Disinfectant Market, 2015 – 2021 (USD Billion)

Source: Zion Research Analysis 2016

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Classes of disinfectants & sanitizers

- Iodophors,
- quaternary ammonium compounds (QAC),
- peroxides,
- phenols,
- chlorine,
- glutaraldehyde

Use of disinfectants

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survey on cleaning & disinfection

- routine cleaning C. diff discharge (52)
- routine cleaning MDRO discharge (52)
- routine cleaning No MDRO (52)
- routine room cleaning CPE present (50)
- routine room cleaning MDRO (50)
- routine outside pt room (53)
- routine cleaning No MDRO (52)

International Survey of cleaning & disinfection practice in the healthcare environment. Venter N, et al. JHI. 2018

France

Incidence, risk factors, and outcome of multidrug-resistant Acinetobacter baumannii acquisition during an outbreak in a burns unit


Antimicrobial Resistance & Environmental Pathogens

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Findings

Prospective study  Burns Unit, Paris- April- Nov 2014
Screening at admission & weekly from patients and environment
25/ 86 (32%) patients acquired MR-AB;  9  pre-colonised (total= 34)
Room environment colonization in 25/34 (74%).
multi locus sequence typing : MR-AB (ST2) found
in 94% patients and 92% environmental strains, all had blaOXA-23 gene.
Of the 25 patients acquiring MR-AB colonization during hospitalization, MR-AB strains were isolated in clinical samples before the environment in 15/25 (60%) patients.

Acinetobacter susceptibility to abt & disinfectants

• 238 clinical isolates of Acinetobacter spp tested against chlorhexidine gluconate, benzethonium chloride, gentamicin, amikacin, acriflavine, tetracycline and ethidium bromide, alkylidiaminoethylglycine hydrochloride (10% w/v), ceftazidime, imipenem, and ciprofloxacin
• 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.
• DRS isolates tended to demonstrate multi resistance profiles to ceftazidime, ciprofloxacin and amikacin (P,0.05).
Acinetobacter susceptibility to abt & disinfectants

Table 3. Correlation between MICs of disinfectants and antimicrobial agents

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Category</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></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>13</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>23</td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>IPM</td>
<td>S</td>
<td>268</td>
<td>0.5</td>
<td></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></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>27</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>AMK</td>
<td>S</td>
<td>268</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>4</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>11</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

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

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

Chlorhexidine, K pneumoniae & colistin

- Adaptation to chlorhexidine resistance led to colistin resistance in 5 of 6 strains of K pneumoniae.
- Mechanism: mutation of regulator genes (smvR) next to MFS regulatory efflux pump smvA gene.
- The phoPQ (from CHG adapted strain) insertion into K pneumoniae resulted in colistin resistance but not chlorhexidine resistance.

**Table 3** MIC and MBC values of CHD and CST after plasmid complementation**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Description</th>
<th>MIC (mg/liter)</th>
<th>MBC (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pACYC-184 alone</td>
<td>Empty vector</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>pACYC M3 smvR WT</td>
<td>SmvR from strain M3</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>pACYC M3 smvR CHD</td>
<td>SmvR from strain M3 CA</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>pACYC 13443 smvR WT</td>
<td>SmvR from strain NCTC 13443</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>pACYC 13443 smvR CHD</td>
<td>SmvR from strain NCTC 13443 CA</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>M109 CA</td>
<td>pACYC-184 phoPQ WT</td>
<td>PhoPQ from strain NCTC 13443</td>
<td>32–64</td>
<td>32–64</td>
</tr>
<tr>
<td></td>
<td>pACYC-184 phoPQ A20P</td>
<td>PhoPQ from strain NCTC 13443</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>M109 WT</td>
<td>pACYC-184 alone</td>
<td>Empty vector</td>
<td>8–16</td>
<td>8–16</td>
</tr>
<tr>
<td></td>
<td>pACYC-184 phoPQ WT</td>
<td>PhoPQ from strain NCTC 13443</td>
<td>0.5–1</td>
<td>2–4</td>
</tr>
<tr>
<td></td>
<td>pACYC-184 phoPQ A20P</td>
<td>PhoPQ from strain NCTC 13443</td>
<td>16–32</td>
<td>32–64</td>
</tr>
<tr>
<td>25</td>
<td>pACYC-184 alone</td>
<td>Empty vector</td>
<td>8–16</td>
<td>8–16</td>
</tr>
<tr>
<td></td>
<td>pACYC-184 phoPQ WT</td>
<td>PhoPQ from strain NCTC 13443</td>
<td>0.5–1</td>
<td>2–4</td>
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<tr>
<td></td>
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<td>PhoPQ from strain NCTC 13443</td>
<td>16–32</td>
<td>32–64</td>
</tr>
</tbody>
</table>

*Levels of resistance to CHD and CST were measured after electroporation of the plasmids into the strains listed. All the MICs are shown as ranges of the results of at least three independent experiments.
C. auris - the new kid on the block

Environmental Surfaces in Healthcare Facilities are a Potential Source for Transmission of Candida auris and Other Candida Species

Christina T. Piedrahita, BS; Jennifer L. Cadnum, BS; Annette L. Jenson, CIC; Aaron A. Shaikh, BS; Mahmoud A. Ghannoum, PhD, FIDSA; Curtis J. Donskey, MD

Infect Control Hosp Epidemiol 2017;38:1107–1109

Recovery rates of C. auris from environment (lab)

Recovery rates of C. auris at 1d and 7d > C. albicans but < C. parapsilosis (P<.05).

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Survival of *C. auris* on surfaces in HCF (clinical)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Dry (N=397)</th>
<th>Moist (N=55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida</td>
<td>16%</td>
<td>4%</td>
</tr>
<tr>
<td>MRSA</td>
<td>2%</td>
<td>4%</td>
</tr>
<tr>
<td>VRE</td>
<td>2%</td>
<td>4%</td>
</tr>
<tr>
<td>FQ-resistant gram-negative bacilli</td>
<td>2%</td>
<td>4%</td>
</tr>
</tbody>
</table>

Sinks and hand wash basins!

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Spain

Control of endemic multidrug-resistant Gram-negative bacteria after removal of sinks and implementing a new water-safe policy in an intensive care unit


Crude rates reduction of MDRO

Post intervention period - reduction in Pseudomonas & Klebsiella

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Biofilm

• Challenge in food industry, home and healthcare
• Within the biofilm
  – Cells within an exopolymeric matrix out-survive those on the surface of the biofilm
  – Repeated chronic exposure to sub-lethal treatments select for a resistant population that share this resistance with other microbes.
  – Gene SigB is activated in both static and continuous biofilm production in wild strains and is involved in planktonic cells and biofilm resistance to peracetic acid & benzalkonium chloride
  – The biocide concentration is strongly affected by the reduced diffusion of active molecules through the biofilm (Anderson and O'Toole 2008, Lewis 2008, Maillard 2007, Tart and Wozniak 2008).

Selection pressure - AWD

• Exposure to benzalkonium chloride (a QAC) showed a population shift and a selection of Pseudomonas spp following treatment.
• Emergence of 2% glutaraldehyde resistant Mycobacterium chelonae
• Gram positive bacteria isolated from AWD following a high level disinfection process using chlorine dioxide.
• The low concentration of the disinfectant or the presence of biofilms (Babb 1993, Pajkos et al. 2004, Smith and Hunter 2008), determine the reduced susceptibility to biocides

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Triclosan
Uses: household products- toothpaste, soap, lotions

Resistance reported
- *S aureus*- cross resistance with mupirocin- *mup gene*
- *E. coli*. ARC ab flux pumps- active transport of disinfectants and antibiotics. MDR
- Pseudomonas spp: expression of the MexAB-OprM efflux system. Selected high frequency MDR. *cip r*
- Acinetobacter spp- efflux pumps
- *M smegmatis*: *Fab1 gene* insusceptibility to triclosan and isoniazid and vice versa
- *Proteus mirabilis* emerging triclosan resistance

How much disinfectants are found in waterways

- 84% of antimicrobial bar soaps contain Triclocarbarn using as high as 750 metric tonnes/year (USA)
- Triclosan in 57% of the 139 waterways tested nationwide (USA).
- High levels found in effluent from hospitals and waterways.
- Primary biodegradation in aerobic soil gave a half-life for triclosan of 18 days!
- In the presence of hypochlorite or sunlight converts to dioxins and other toxic compounds
- Absorbed in human (mothers milk) and aquatic life (rainbow trout).
- Sweden abandoned triclosan use in hospitals

PhD thesis 2012. M Alshuli, Phil.USA
MRSA

- Mupirocin resistance in MRSA strains carried a **quaternary ammonium resistance** gene (*qacA*) located in a gentamicin resistance plasmid that encoded for an efflux mechanism resulting in low-level chlorhexidine resistance.
- **Transferable triclosan** resistance in MRSA has been described, occurring together with a high-level of mupirocin in a hospital environment.

Quaternary Ammonium compounds (QAC)

Uses: household, cosmetics, perfume

- 238 Clinical b/c isolates of *S. aureus* and *S. epidermidis* from children
- In 78 BC* staphylococcal isolates, resistance to QAC- 50%
- **plasmids**- *qacA* or *B* (*qacA/B*), *qacC*, *blaZ*, and *tetK*
- Qac linkage between disinfectants and penicillins in clinical isolates in Norway

Acne treatment
Inactive ingredients, benzalkonium chloride, other QAC.


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Mechanisms of resistance to biocides

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>Nature</th>
<th>Level of susceptibility to other biocides</th>
<th>Cross-resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeability</td>
<td>intrinsic (acquired)</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Efflux</td>
<td>intrinsic/acquired</td>
<td>reduced</td>
<td>yes</td>
</tr>
<tr>
<td>Degradation</td>
<td>acquired/intrinsic</td>
<td>reduced</td>
<td>no</td>
</tr>
<tr>
<td>Mutation (target site)</td>
<td>acquired</td>
<td>reduced</td>
<td>no</td>
</tr>
<tr>
<td>Phenotypic change</td>
<td>Following exposure</td>
<td>reduced</td>
<td>yes</td>
</tr>
<tr>
<td>Induction (stress response)</td>
<td>Following exposure</td>
<td>variable</td>
<td>yes</td>
</tr>
</tbody>
</table>

1 to other biocides - level of susceptibility defined according to the concentration of biocides
2 not to other biocide, but cross-resistance with specific antibiotics

The induction of bacterial resistance has been described in almost all biocides, such as quaternary ammonium compounds, bisbiguanides and phenolics, as well as glutaraldehyde.

Regulations adopted 2009

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Measuring resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance to a biocide</td>
<td>Change in phenotypes</td>
</tr>
<tr>
<td>MBCs</td>
<td>Yes</td>
</tr>
<tr>
<td>Bactericidal activity</td>
<td>Yes</td>
</tr>
<tr>
<td>Inactivation kinetic</td>
<td>Yes</td>
</tr>
<tr>
<td>MICs</td>
<td>No*</td>
</tr>
<tr>
<td>Growth kinetic</td>
<td>No*</td>
</tr>
</tbody>
</table>

* An increase in MIC might provide information about a trend towards insusceptibility
**Contamination of disinfectants (HCF)**

- *Ps aeruginosa* from iodophors - failed manufacturing
- *Serratia marcescens* from contaminated QAC & CHG
- *Burkholderia cepacia* from multi use disinfectants
- *Ps aeruginosa* resistance to metal such as silver- silver nitrate dressings
- *Ps aeruginosa* isolated from cosmetics and several other types of products is pathogenic and resistant to several types of antibiotics (Scully et al. 1986).

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**Chlorine & antimicrobial resistance**

22 genera were isolated from chlorinated drinking-water with a range of susceptibilities to chlorine and antibiotics.

- Chlorine-resistant bacteria had higher MICs for tetracycline, sulfamethoxazole and amoxicillin.
- In the presence of free chlorine, antibiotic-sensitive bacteria survival was less than antibiotic-resistant bacteria.

Weak correlations were found between chlorine-tolerance and minimum inhibitory concentrations against the antibiotics tetracycline, sulfamethoxazole and amoxicillin (transmissible genes) but not against ciprofloxacin (efflux pumps and porins) so most likely not on the *mac* operon.

Antibiotic-resistant bacteria survived longer than antibiotic-sensitive organisms when exposed to free chlorine in a contact-time assay.

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Chemosphere, 2016: 152

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Deliberate exposure to chlorine spraying - Ebola

<table>
<thead>
<tr>
<th>Site</th>
<th>HCW</th>
<th>EVO</th>
<th>NEVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total interviewed</td>
<td>500</td>
<td>550</td>
<td>500</td>
</tr>
<tr>
<td>Not sprayed (excluded)</td>
<td>7</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Total analysed</td>
<td>N = 493</td>
<td>N = 550</td>
<td>N = 477</td>
</tr>
<tr>
<td>In own house (under quarantine)</td>
<td>2</td>
<td>2</td>
<td>440</td>
</tr>
<tr>
<td>Outside in the community</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Pre transfer</td>
<td>0</td>
<td>162</td>
<td>39</td>
</tr>
<tr>
<td>Back of ambulance</td>
<td>61</td>
<td>12</td>
<td>99</td>
</tr>
<tr>
<td>Leaving ETU</td>
<td>550</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Red zone</td>
<td>455</td>
<td>93</td>
<td>8</td>
</tr>
<tr>
<td>Spray others</td>
<td>113</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>In room when spraying others</td>
<td>116</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>EVO case house</td>
<td>16</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>EVO suspect house</td>
<td>33</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2** Chlorine spraying in the three groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Single Cl₂ exposure (N = 285) n(%,)</th>
<th>Multiple Cl₂ exposure (N = 208) n(%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye sight problem before</td>
<td>19 (7)</td>
<td>25 (12)</td>
<td>0.04</td>
</tr>
<tr>
<td>Eye sight problem now</td>
<td>95 (34)</td>
<td>123 (59)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coughing</td>
<td>107 (38)</td>
<td>124 (60)</td>
<td>0.001</td>
</tr>
<tr>
<td>Coughing producing sputum</td>
<td>43 (15)</td>
<td>60 (29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Difficulty in breathing</td>
<td>66 (23)</td>
<td>100 (48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chest tightness</td>
<td>109 (38)</td>
<td>131 (63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Burning throat</td>
<td>85 (30)</td>
<td>112 (54)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Skin irritation</td>
<td>95 (34)</td>
<td>109 (52)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

HCW = healthcare workers; EVO = Ebola virus disease survivors; NEVD = non-Ebola cases

163 staff interviewed; 49 air samples taken

Health problems and disinfectant product exposure among staff at a large multispecialty hospital

<table>
<thead>
<tr>
<th>Symptom</th>
<th>All participants (N = 161)</th>
<th>Work-related symptoms*</th>
<th>Disinfectant product use (n = 78)</th>
<th>No disinfectant product use (n = 85)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal problems⁰</td>
<td>68 (42)</td>
<td>20 (18)</td>
<td>31 (40)</td>
<td>37 (44)</td>
<td>.64</td>
</tr>
<tr>
<td>Watery eyes⁰</td>
<td>65 (40)</td>
<td>31 (18)</td>
<td>35 (45)</td>
<td>30 (35)</td>
<td>.26</td>
</tr>
<tr>
<td>Asthma-like symptoms⁰</td>
<td>46 (28)</td>
<td>16 (10)</td>
<td>24 (31)</td>
<td>22 (26)</td>
<td>.60</td>
</tr>
<tr>
<td>Skin problems⁰</td>
<td>31 (19)</td>
<td>19 (11)</td>
<td>12 (15)</td>
<td>19 (22)</td>
<td>.32</td>
</tr>
<tr>
<td>Wheeze⁰</td>
<td>26 (16)</td>
<td>6 (4)</td>
<td>11 (15)</td>
<td>14 (16)</td>
<td>.99</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>27 (17)</td>
<td>7 (4)</td>
<td>11 (14)</td>
<td>10 (12)</td>
<td>.82</td>
</tr>
<tr>
<td>Chest tightness⁰</td>
<td>18 (11)</td>
<td>4 (2)</td>
<td>10 (13)</td>
<td>8 (9)</td>
<td>.62</td>
</tr>
<tr>
<td>Cough</td>
<td>9 (6)</td>
<td>4 (2)</td>
<td>5 (6)</td>
<td>4 (5)</td>
<td>.74</td>
</tr>
<tr>
<td>Asthma attack⁰</td>
<td>8 (5)</td>
<td>4 (2)</td>
<td>1 (4)</td>
<td>5 (6)</td>
<td>.72</td>
</tr>
<tr>
<td>Medication use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergy medicine</td>
<td>48 (29)</td>
<td>9 (6)</td>
<td>16 (21)</td>
<td>32 (38)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Asthma medicine</td>
<td>10 (11)</td>
<td>6 (4)</td>
<td>10 (13)</td>
<td>8 (9)</td>
<td>.62</td>
</tr>
</tbody>
</table>
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Fig 1. Prevalence of work-related symptoms by disinfectant product use at a hospital, August 2015. Work-related symptoms were defined as symptoms that improved away from the facility, either on days off or on vacation. *Statistically significant differences using χ² test (P < 0.05). All symptoms specific to the last 12 months.

Take home message

Disinfectants

✓ Sub-lethal doses
✓ Inadequate removal
✓ Inappropriate use

Antimicrobial resistance

It all boils down to removing the biofilm!
The Impact of Disinfectants on Antimicrobial Resistance
Prof. Shaheen Mehtar, Stellenbosch University, Cape Town, South Africa
Broadcast live from the 2018 Infection Prevention Society Conference

Prof Ayliffe- A mentor to many

Prof Graham Ayliffe
1926-2017
A gentleman and a scholar
who taught me to think!

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<th>Date</th>
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| October 11, 2018 | **FREE CBIC Teleclass**<br>INFECTION CONTROL CHAMPIONS ARE MADE, NOT BORN  
Speaker: To be announced |
| October 17, 2018 | **South Pacific Teleclass**<br>BIOFILMS IN THE HOSPITAL ENVIRONMENT - INFECTION CONTROL  
IMPLICATIONS  
Speaker: Prof. Karen Vickery, Macquarie University, Australia |
| October 18, 2018 | **FREE Teleclass**<br>INFECTION PREVENTION CORE PRACTICES: RESETTING THE BAR FOR  
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