The affect of pH and bacterial phenotypic state on antibiotic efficacy

John Thomas, Sara Linton, Linda Corum, Will Slone, Tyler Okel, Steven L Percival

ABSTRACT

Antibiotics are routinely used in wound care for the treatment of local and systemic infections. Our goals in this paper were to (i) evaluate the antibiotic sensitivity of bacteria isolated from burn and chronic wounds and (ii) evaluate the effect of pH and bacterial phenotype on the efficacy of antibiotics. Chronic and burn wound isolates, which had been routinely isolated from patients at West Virginia University Hospital, USA, were evaluated for their sensitivity to antibiotics. Antimicrobial susceptibility testing was performed using a standardised disk diffusion assay on agar (quasi/non biofilm) and poloxamer (biofilm). Many of the Gram-positive and -negative isolates demonstrated changes in susceptibility to antibiotics when grown at different pH values and phenotypic states. Findings of this study highlight the clinical relevance that both pH and the phenotypic state of bacteria have on antibiotic performance. The study in particular has shown that bacteria exhibit an enhanced tolerance to antibiotics when grown in the biofilm phenotypic state. Such a finding suggests that more appropriate antibiotic sensitivity testing for wound care and medicine is warranted to help assist in the enhancement of positive clinical outcomes.

Key words: Antibiotics • Biofilms • Silver • Wounds • Wound dressings

INTRODUCTION

Within the wound environment, microorganisms have been reported to reside in both a planktonic and sessile (biofilm) state, with the recalcitrant biofilm state considered the most significant to delayed wound healing (1,2). Biofilms are composed of microbial cells which are encased within a matrix of extracellular polymeric substances (EPS) and attached to each other, or to a non biological or biological surface/support. Bacteria which reside in the biofilm phenotypic state are known to differ significantly from their planktonic counterparts (3). Consequently, antibiotics and other antimicrobials used in the management of at-risk or infected wounds must show efficacy on microorganisms residing in both the planktonic and sessile states.

Antibiotics are used for a range of purposes but specifically for preventing or limiting a local or systemic infection and are routinely administered, as part of a treatment regime, for both bacterial and fungal infections in acute and chronic wounds.

Key Points

- antibiotics are used for a range of purposes but specifically for preventing or limiting a local or systemic infection and are routinely administered, as part of a treatment regime, for both bacterial and fungal infections in acute and chronic wounds
is a growing concern worldwide on the failure of antibiotic therapies and the increasing emergence of antibiotic resistance in bacteria. Such antibiotic therapy failures have been reported to have arisen for a number of reasons including the overuse of antibiotics (4), inappropriate use in many human and animal conditions (5–7), inadequate or incorrect prescribing and non completion of a prescribed course of antibiotics by patients.

Many factors are known to affect the activity of antibiotics and antiseptics and have included pH (8), temperature, concentration, contact time, biological matter and the microbial phenotypic state (3,9). As an example it has been found that the activity of macrolides and aminoglycosides are reduced in alkaline conditions (10,11). Contrary to this the activity of beta-lactam antibiotics are increased on bacteria in acidic conditions (12). A similar effect of pH on the activity of antiseptics has been shown. For example, chlorhexidine and quaternary ammonium compounds (QACs) are known to be more active at an alkaline rather than an acidic pH (13).

In addition to pH and bacterial phenotype affecting the activity and choice of antimicrobial for the management of infected wounds there is a growing concern that the overuse of some antiseptics may lead to cross resistance to certain antibiotics. Such a phenomena has been documented for an array of different antiseptics. For example, Akimitsu and colleagues (14) found that meticillin-resistant Staphylococcus aureus (MRSA) mutants that were resistant to benzalkonium chloride also exhibited increased resistance to a number of beta-lactam antibiotics. Further to this colleagues (14) found that meticillin-resistant Staphylococcus aureus (MRSA), meticillin-sensitive Staphylococcus aureus (MSSA), Streptococcus sp, Enterococcus sp and Gram-negative bacteria: Pseudomonas aeruginosa, Acinetobacter sp and Gram-negative rods.

**Key Points**

- There is a growing concern worldwide on the failure of antibiotic therapies and the increasing emergence of antibiotic resistance in bacteria.
- To help reduce the risk of cross resistance occurring between antibiotics and antiseptics appropriate usage of both these agents is warranted in clinical practice.
- Our goals in this paper were to (i) evaluate the antibiotic sensitivity of bacteria isolated from burn and chronic wounds and (ii) evaluate the effect of pH and bacterial phenotype on the efficacy of antibiotics.

**MATERIALS AND METHODS**

**Test micro-organism**

Chronic and burn wound isolates which had been routinely isolated from burn and chronic wound patients were evaluated in this study. The test isolates included Gram-positive bacteria: vancomycin-resistant Enterococcus (VRE), meticillin-resistant Staphylococcus aureus (MRSA), meticillin-sensitive Staphylococcus aureus (MSSA), Streptococcus sp, Enterococcus sp and Gram-negative bacteria: Pseudomonas aeruginosa, Acinetobacter sp and Gram-negative rods.

**Evaluated antibiotics**

The following panel of antibiotics were tested: clindamycin (CC2 – 2 μg), ampicillin (AM10 – 10 μg), aztreonam (ATM30 – 30 μg) and levofloxacin (LVX5 – 5 μg). All antibiotic disks were purchased from Becton-Dickinson (Becton-Dickinson, Sparks, MD, USA). Appropriate standard reference strains were used to enable resistance and sensitivity claims (17).

**Antibiotic efficacy testing**

Each test organism was grown overnight in Tryptone Soy Broth (TSB) and then added to saline (0.85%). The inoculated saline (containing 1 × 10⁶ CFUs/ml) was then swabbed onto Mueller Hinton Agar (MHA), or poloxamer plates, according to Clinical and Laboratory Standards Institute (CLSI) guidelines (17). Each MHA (quasi/non biofilm state) or poloxamer (biofilm) plate was divided into segments to incorporate two specific antibiotic disks of either clindamycin (CC2) and ampicillin (AM10) for Gram-positive isolates, and aztreonam (ATM30) or levofloxacin (LVX5) for Gram-negative isolates.

To show the effect of pH on the activity of antibiotics, MHA and poloxamer plates were made to a pH of 7 or 5.5 (by the addition of hydrochloric acid or sodium hydroxide during media preparation). After incubation the zone of inhibition (ZOI) around each antibiotic disk was recorded. All experiments were carried out in triplicate.
Biofilm test method and procedure

The biofilm test model setup can be located elsewhere (18–20). Briefly, poloxamer (30%) was incorporated into Mueller Hinton Broth (MHB) and refrigerated overnight (4°C). The poloxamer suspension was then autoclaved and returned to the fridge. Liquefied poloxamer was poured into Petri dishes and then incubated overnight at 37°C before inoculation with a test isolate.

Statistical analysis

A Student’s t-test was used to compare zones of inhibition. All data were analysed using Microsoft Excel.

RESULTS

Gram-positive wound isolates – effect of pH

Forty-seven Gram-positive chronic wound isolates, including, VRE, MRSA, β and α haemolytic Streptococcus sp and MSSA were exposed to antibiotics AM10 and CC2 (Figure 1). A zone of clearing around AM10 was noted for all MRSA strains at a pH of 7, with a mean ZOI of 12 mm observed. CC2 was found to be much more efficacious on MRSA strains than AM10. All eight MSSA isolates were found to be sensitive to both antibiotics AM10 and CC2 at a pH of 7 with a mean ZOI recorded at 20.3 and 26 mm, respectively. All three Streptococcus sp were found to be sensitive to CC2 at a pH of 7 with poorer efficacy noted for AM10. All of the 16 VRE strains showed complete resistance to AM10 and CC2 at pH 7.0 according to standard resistance classification guidelines (17).

When a representative number of Gram-positive chronic and burn wound isolates were exposed to antibiotics at a pH of 7 or 5.5 activity was found to be affected (Table 1). Staphylococcus aureus, including MRSA, appeared more susceptible to CC2 at a pH of 7.0 compared with pH 5.5. This however was not significant ($P < 0.05$). The opposite was true for sensitivity to AM10. All chronic wound Enterococcus isolates showed tolerance to AM10 at both pH 5.5 and 7. Interestingly all strains of Enterococcus sp isolated from burn wounds were found to be sensitive to both antibiotics AM10 and CC2.

Gram-negative wounds isolates – effect of pH

At a pH of 7 the efficacy of ATM30 and LVX5 was increased slightly on a representative sample of Gram-negative rods isolated from chronic wounds compared with a pH of 5.5 (Table 2). P. aeruginosa strains isolated from chronic wounds were sensitive to ATM30 with efficacy of the antibiotic increasing slightly at a pH of 7 compared with 5.5 – the opposite was true for the antibiotic LVX5.

Five strains of P. aeruginosa isolated from burn wounds were exposed to ATM30 and LVX5. The average ZOI for ATM30 was

![Figure 1](image_url). The efficacy of antibiotics ampicillin [AM10] and clindamycin [CC2] (mean ZOI [mm]) on Gram-positive bacteria isolated from chronic wounds and grown in the quasi/non biofilm state at a pH of 7.

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The efficacy [mean ZOI (mm)] of antibiotics ampicillin (AM10) and clindamycin (CC2) on a representative sample of Gram-positive wound isolates grown in the quasi/non biofilm state (±SE in brackets) at pH 5-5 and 7

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Wound type</th>
<th>Number</th>
<th>AM10 pH 7 (±SE)</th>
<th>AM10 pH 5-5 (±SE)</th>
<th>CC2 pH 7 (±SE)</th>
<th>CC2 pH 5-5 (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus sp (including VRE)</td>
<td>Burn</td>
<td>7</td>
<td>27.6 ± 1.8</td>
<td>32.9 ± 1.3</td>
<td>9.1 ± 6.5</td>
<td>11.9 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>Chronic</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>2.7 ± 2.7</td>
<td>0</td>
</tr>
<tr>
<td>Staphylococcus aureus (including MRSA)</td>
<td>Burn</td>
<td>4 (3 MRSA)</td>
<td>17.1 ± 9.7</td>
<td>28.4 ± 7.2</td>
<td>22.9 ± 7.6</td>
<td>15.8 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>Chronic</td>
<td>8 (5 MRSA)</td>
<td>11.6 ± 1.0</td>
<td>17.6 ± 3.1</td>
<td>22.8 ± 4.1</td>
<td>13.9 ± 4.1</td>
</tr>
</tbody>
</table>

SE, standard error; ZOI, zone of inhibition.

The efficacy [mean ZOI (mm)] of antibiotics aztreonam (ATM30) and levofloxacin (LVX5) on a representative sample of Gram-negative wound isolates grown at pH 5 and 7 in the quasi/non biofilm state

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Wound type</th>
<th>Number</th>
<th>ATM30 pH 7 (±SE)</th>
<th>ATM30 pH 5-5 (±SE)</th>
<th>LVX5 pH 7 (±SE)</th>
<th>LVX5 pH 5-5 (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative rods</td>
<td>Chronic</td>
<td>4</td>
<td>30.0 ± 3.0</td>
<td>27.3 ± 3.0</td>
<td>31.2 ± 2.0</td>
<td>29.4 ± 4.0</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>Burn</td>
<td>4</td>
<td>8.9 ± 3.0</td>
<td>11.0 ± 4.0</td>
<td>12.5 ± 6.2</td>
<td>7.6 ± 7.6</td>
</tr>
<tr>
<td>Enterobacter sp</td>
<td>Burn</td>
<td>5</td>
<td>27.3 ± 8.5</td>
<td>24.3 ± 7.6</td>
<td>26.2 ± 8.5</td>
<td>18.3 ± 6.8</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Burn</td>
<td>6</td>
<td>33.1 ± 1.0</td>
<td>28.7 ± 1.2</td>
<td>28.9 ± 7.0</td>
<td>18.3 ± 8.0</td>
</tr>
<tr>
<td>Klebsiella sp</td>
<td>Burn</td>
<td>4</td>
<td>33.4 ± 0.8</td>
<td>20.1 ± 7.1</td>
<td>31.8 ± 0.7</td>
<td>22.1 ± 1.6</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>Burn</td>
<td>3</td>
<td>41.0 ± 0.3</td>
<td>41.0 ± 1.7</td>
<td>30.3 ± 7.8</td>
<td>31.2 ± 9.1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Chronic</td>
<td>2</td>
<td>18.6 ± 6.0</td>
<td>14.25 ± 14.0</td>
<td>12 ± 12.0</td>
<td>14 ± 14.0</td>
</tr>
<tr>
<td></td>
<td>Burn</td>
<td>5</td>
<td>19.6 ± 1.4</td>
<td>19.8 ± 5.0</td>
<td>18.4 ± 2.2</td>
<td>18.1 ± 5.0</td>
</tr>
</tbody>
</table>

SE, standard error; ZOI, zone of inhibition.

calculated to be 19.6 mm at a pH of 7 and 19.8 mm at a pH of 5.5. For LVX5 the zones of inhibition were very similar at pH of 7 and 5.5.

Four strains of Acinetobacter baumannii isolated from burn wounds showed a slight increased sensitivity to ATM30 at a pH of 5.5 compared with a pH of 7. However, the opposite was true for LVX5. For all Enterobacter sp, E. coli and Klebsiella sp screened against ATM30 and LVX5 the activity of both antibiotics was enhanced at a pH of 7 compared with a pH of 5.5. However, this increased sensitivity was not significant (P < 0.05). Proteus mirabilis isolates showed equal sensitivity to ATM30 and LVX5 at both pH of 7 and 5.5.

Gram-positive wound isolates – effect of phenotypic state

All Gram-positive bacteria, apart from one strain of S. aureus isolated from chronic wounds, were found to have a slightly increased tolerance to AM10 and CC2 when grown in the biofilm state compared with the quasi/non biofilm state (Table 3). This effect was however not found to be significant (P < 0.05). The mean CZOI for the burn wound Enterococcus isolates was found to be significantly larger (P < 0.05) for the antibiotic AM10 when the isolates were grown in the quasi/non biofilm state and compared with the mean CZOI in the biofilm state. For the chronic wound MRSA isolates the efficacy of CC2 was significantly reduced (P < 0.05) when the bacteria were grown in the biofilm state compared with growth in the quasi/non biofilm state.

Gram-negative wound isolates – effect of phenotypic state

P. aeruginosa strains isolated from both chronic and burn wounds showed an increased tolerance to ATM30 when grown in the biofilm phenotypic state (Table 4). These results however were found not to be significant. For the antibiotic LVX5 enhanced tolerance in the
biofilm state was not showed for the chronic wound isolates. For all other Gram-negative strains, isolated from chronic wounds, tolerance to both ATM30 and LVX5 when grown in the biofilm phenotypic state was showed when compared with growth in the quasi/non biofilm state.

For the burn isolates Enterobacter sp, E. coli, Klebsiella sp, Pseudomonas sp and Proteus sp the mean zones of inhibition around antibiotics ATM30 and LVX5 were overall smaller when grown in the biofilm phenotypic state compared with the quasi/non biofilm state.

When Acinetobacter baumannii was grown in the non biofilm state and compared with growth in the biofilm phenotypic state both ATM30 and LVX5 exhibited similar efficacies irrespective of phenotypic state.

Overall, increased tolerance to antibiotics in the biofilm phenotypic state was showed to be significantly different ($P < 0.05$) for Enterococcus sp (ATM30), E. coli (ATM30 and LVX5), Klebsiella sp (ATM30 and LVX5) and Proteus sp (ATM30).

**DISCUSSION**

Wounds are known to harbour a complex population of microorganisms which create and maintain conditions in the wound that prevent them from healing in a timely manner (21). To help reduce the microbiological colonisation of a wound, systemic and topical antibiotics are often administered (22).

In this study we evaluated chronic and burn wound isolates for their sensitivity to commonly used antibiotics administered in woundcare. The main goal was to determine whether the activity of antibiotics was affected by both pH and bacterial phenotypic state.

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**Table 3** A comparison of the efficacy [mean ZOI (mm)] of antibiotics ampicillin (AM10) and clindamycin (CC2) on Gram-positive chronic and burn wound isolates grown in the quasi/non biofilm and biofilm phenotypic states

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Wound type</th>
<th>Number</th>
<th>Non biofilm (±SE)</th>
<th>Biofilm (±SE)</th>
<th>Non biofilm (±SE)</th>
<th>Biofilm (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus sp</td>
<td>Burn</td>
<td>7</td>
<td>27.6 ± 1.7</td>
<td>17.7 ± 3.2*</td>
<td>9.1 ± 4.7</td>
<td>6.4 ± 4.0</td>
</tr>
<tr>
<td>Enterococcus sp (VRE)</td>
<td>Chronic</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5.1 ± 5.1</td>
<td>2.9 ± 2.9</td>
</tr>
<tr>
<td>Staphylococcus aureus (3 MRSA)</td>
<td>Burn</td>
<td>4</td>
<td>17.1 ± 7.9</td>
<td>13.1 ± 6.4</td>
<td>22.9 ± 7.6</td>
<td>15.6 ± 1.4</td>
</tr>
<tr>
<td>MRSA</td>
<td>Chronic</td>
<td>5</td>
<td>11.5 ± 1.4</td>
<td>4.8 ± 2.0</td>
<td>26.1 ± 2.2</td>
<td>12.1 ± 3.1*</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Chronic</td>
<td>2</td>
<td>11.0 ± 1.0</td>
<td>12.0 ± 1.3</td>
<td>14.5 ± 14.5</td>
<td>7.3 ± 7.3</td>
</tr>
</tbody>
</table>

SE, standard error; ZOI, zone of inhibition.
*Significantly different at 95% confidence interval.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Wound type</th>
<th>Number</th>
<th>AM10 Non biofilm (±SE)</th>
<th>AM10 Biofilm (±SE)</th>
<th>CC2 Non biofilm (±SE)</th>
<th>CC2 Biofilm (±SE)</th>
</tr>
</thead>
<tbody>
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<td>Burn</td>
<td>4</td>
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<td>26.9 ± 6.7</td>
<td>15.7 ± 4.1*</td>
<td>25.5 ± 7.3</td>
<td>20.7 ± 0.9</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Burn</td>
<td>6</td>
<td>33.1 ± 1.0</td>
<td>19.1 ± 0.4*</td>
<td>28.9 ± 7.0</td>
<td>19.5 ± 3.0*</td>
</tr>
<tr>
<td>Klebsiella sp</td>
<td>Burn</td>
<td>4</td>
<td>31.8 ± 0.7</td>
<td>21.5 ± 1.8*</td>
<td>33.4 ± 0.8</td>
<td>24.8 ± 1.4*</td>
</tr>
<tr>
<td>Proteus sp</td>
<td>Burn</td>
<td>3</td>
<td>41.0 ± 0.3</td>
<td>22.0 ± 0.8*</td>
<td>30.3 ± 7.8</td>
<td>19.5 ± 2.2</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Burn</td>
<td>5</td>
<td>19.6 ± 1.4</td>
<td>13.9 ± 0.8</td>
<td>18.4 ± 2.2</td>
<td>17.0 ± 2.1</td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td>Chronic</td>
<td>2</td>
<td>18.0 ± 6.0</td>
<td>13.5 ± 3.0</td>
<td>12.0 ± 12.0</td>
<td>14.3 ± 6.3</td>
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<td>Gram-negative rods</td>
<td>Chronic</td>
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</table>

SE, standard error; ZOI, zone of inhibition.
*Significantly different at 95% confidence interval.
Firstly the sensitivity of Gram-positive bacteria to CC2 and AM10 was evaluated. AM10 and CC2 are used extensively in the treatment of bacterial infections caused by Gram-positive organisms such as, staphylococci and streptococci specifically, although AM10 has showed activity against Gram-negative organisms such as coliforms and Proteus sp (23). ATM30 and LVX5 were evaluated for their effectiveness on Gram-negative isolates. ATM30 is a synthetic monocyclic beta-lactam antibiotic that has strong activity against Gram-negative bacteria, including P. aeruginosa, Citrobacter sp, Enterobacter sp, E. coli, Klebsiella sp, Proteus sp and Serratia sp (24). However, it has been shown to have poor, if any, activity on Gram-positive bacteria. LVX5 is a broad-spectrum antibiotic that has been found to be active against both Gram-positive and Gram-negative bacteria.

The majority of studies that have evaluated the antimicrobial performance of antibiotics and antimicrobials have, in general, only investigated their ability to prevent the growth of quasi/non biofilm or planktonic microorganisms. Consequently, in this study we wanted to evaluate performance of antibiotics on bacteria grown in the biofilm phenotypic state. ATM30 was evaluated. AM10 and LVX5 were evaluated for their effectiveness on Gram-negative isolates. ATM30 is a synthetic monocyclic beta-lactam antibiotic that has strong activity against Gram-negative bacteria, including P. aeruginosa, Citrobacter sp, Enterobacter sp, E. coli, Klebsiella sp, Proteus sp and Serratia sp (24). However, it has been shown to have poor, if any, activity on Gram-positive bacteria. LVX5 is a broad-spectrum antibiotic that has been found to be active against both Gram-positive and Gram-negative bacteria.

The study found that the effectiveness and sensitivity of bacteria to antibiotics was affected slightly by both pH and bacterial phenotype. In particular, it was found that the activity of ampicillin on MRSA grown in the quasi/non biofilm phenotypic state increased when pH was decreased to 5.5 compared with a pH of 7.0. In addition many bacteria grown within the biofilm phenotypic state showed enhanced tolerance to antibiotics when compared with their planktonic/non-biofilm counterparts.

In vitro antimicrobial susceptibility testing is very important in medicine and woundcare and is used to help predict the in vivo success or failure of antibiotic therapy. The in vitro testing of the efficacy of an antibiotic is performed under standardized conditions following appropriate guidelines to ensure that the results are reproducible and useful. All in vitro results are interpreted on available CLSI (17) data. The results generated help to guide antibiotic choice. However, any in vitro antimicrobial susceptibility testing should be combined with clinical information and experience when selecting the most appropriate antibiotic for a patient. The susceptibility of bacteria to a specific antibiotic implies that the isolate is inhibited by the usually achievable concentrations of antimicrobial agent. However, this definition says nothing about the chances of clinical success. The resistance of an isolate to an antibiotic suggests that it is inhibited by the usual concentrations of the antibiotic used. The zone diameters generated have to fall within a certain range where specific microbial resistance mechanisms are likely to occur, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies. These ranges are specified by the CLSI(17) and updated regularly. Therefore within this study whilst a small number of isolates exhibited very small ZOI these isolates were still classified as resistant under present guidelines. However, unlike antibiotic sensitivity testing for planktonic and quasi/non biofilm bacteria, appropriate guidelines and standards for evaluating antibiotics against biofilms do not exist. Consequently, such an approach is required to help guide appropriate antibiotic usage for those infections known to be biofilm related.

Overall, findings of this study highlight the clinical relevance that both pH and the phenotypic state of bacteria have on antibiotic performance. The study has showed that bacteria exhibit an enhanced tolerance to antibiotics when grown in the biofilm phenotypic state. Such a finding suggests that more appropriate antibiotic sensitivity testing for woundcare and medicine in general is warranted to help assist in the enhancement of positive clinical outcomes. Whilst the in vitro models and conditions used in this study...

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**Key Points**

- The majority of studies that have evaluated the antimicrobial performance of antibiotics and antimicrobials have, in general, only investigated their ability to prevent the growth of quasi/non biofilm or planktonic microorganisms.
- Consequently, in this study we wanted to evaluate the performance of antibiotics on bacteria grown in the biofilm phenotypic state.
- The study found that the effectiveness and sensitivity of bacteria to antibiotics was affected slightly by both pH and bacterial phenotype.
- In particular, it was found that the activity of ampicillin on MRSA grown in the quasi/non biofilm phenotypic state increased when pH was decreased to 5.5 compared with a pH of 7.0.
- In addition many bacteria grown within the biofilm phenotypic state showed enhanced tolerance to antibiotics when compared with their planktonic/non-biofilm counterparts.
- Also, the study has showed that all antibiotics exhibited variations in their activity against species and genera of chronic and burn wound bacteria.
- In particular it was found that for the Gram-negative isolates antibiotics remained efficacious.
- The study has showed that bacteria exhibit an enhanced tolerance to antibiotics when grown in the biofilm phenotypic state.
- Such a finding suggests that more appropriate antibiotic sensitivity testing for wound care and medicine in general is warranted to help assist in the enhancement of positive clinical outcomes.

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were remote from those prevailing in a patient with a chronic or burn wounds the results achieved may nevertheless help to further enhance our present understanding of how the activity of antibiotics could be improved for clinical practices. Strategies are warranted to help increase the efficacy of antibiotics on the microbial bioburden and infection in wounds. Consequently, based on the findings in this study it appears that if we adopt more robust in vitro biofilm models which are more appropriate and relevant to the in vivo situation, and investigate the variables that affect antimicrobial performance better clinical outcomes for the patient could potentially be achieved.

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