The in vitro inhibitory effect of ox bile on selected bacteria

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The minimum inhibitory concentration (MIC) end points of ox bile against selected species of bacteria

Salah El Din Abdel Hag¹, Sania A. Shaddad²*, Tigani Hassan³, Sumaya I Abass⁴, A.K. Muddathir⁵ & Shayoub M. E. A⁶.

Affiliation:-
¹Department of Pharmacology, College of Medicine, University of Bahr Elghazal, Sudan
²Department of Pharmacology, Faculty of Medicine, University of Khartoum, Sudan
³Department of Medicine, Pharmacology & Toxicology, Faculty of Veterinary Medicine University of Khartoum, Sudan
⁴Microbiology-Veterinary Research Centre, Khartoum Sudan
⁵Department of Pharmacognosy, Faculty of Pharmacy, University of Khartoum, Sudan
⁶Department of Pharmaceutics, Faculty of Pharmacy, University of Khartoum, Sudan

*Corresponding author:
Dr. Sania A. Shaddad
Department of Pharmacology, Faculty of Medicine, University of Khartoum, Sudan.

Abstract:
Pathogenic isolates of bacteria were cultured using standard cultivation techniques. Fresh ox bile was collected & dilution prepared under aseptic conditions. The minimum inhibitory concentration (MIC) end points of ox bile on the selected microorganisms were determined using standard microbiologic methods. It was 6.25 % ox bile to Staphylococcus albus, Corynebacterium pseudotuberculosis, Staphylococcus saprophyticus, and Micrococcus variant, 50 % to E. coli, Pseudomonas spp and Klebsiella spp, and 100 % (bile ‘as such’) to Staphylococcus albus, Escherichia vulneris, Staphylococcus aureus, Proteus spp. and Bacillus spp. Values showed variation with the variation in the cultivation technique. The results are suggestive of bactericidal action.

Key Words: MIC; Ox bile; bactericidal.

INTRODUCTION

The minimum inhibitory concentration (MIC) defined as the minimum concentration, of an antimicrobial, that completely inhibits the growth of a microorganism as determined for ox bile against the in vitro growth of Staphylococcus albus, Proteus spp., Bacillus spp, Staphylococcus aureus, Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa, Corynebacterium pseudotuberculosis, Escherichia vulneris, Bacillus subtilis (Chemotherapeutic sensitive), Staphylococcus saprophyticus; Enterobacter spp.; and Micrococcus variant.

It had been repeatedly shown that the optimum antibacterial effect under clinical situations can be generated by administering an antibacterial, for a 24 hour dose interval, with a peak concentration 8~10 times its MIC, or a AUC: MIC ratio of 125~250. The demonstration of the sensitivity of these bacteria to bile of the ox, and the observation that the bacteria subjected to testing were indifferently sensitive to 100 % of the substance (bile ‘as such’) together with the need to about the likely chemotherapeutic utility of the substance provoked the conduction of the present
study with a view to determine the MIC end points.

MATERIALS & METHODS

**Bacteria;**
Pathogenic isolates of strains of *Staphylococcus albus, Proteus spp, Bacillus Gm+ve spp, Staphylococcus aureus, Escherichia coli, Klebsiella spp, Pseudomonas aeruginosa, Corynebacterium pseudotuberculosis, Escherichia vulneris, Bacillus subtilis* (Chemotherapeutic sensitive), *Staphylococcus saprophyticus, Enterobacter spp.* and *Micrococcus variant* were kindly supplied by Department of Bacteria, Central Veterinary Research Laboratories Centre, Soba, Sudan.

**Bile collection;**
Fresh ox bile samples were collected (twice a week) during the post-mortem inspection at Sabaloka abattoir, Omdurman, Sudan; 3ml of bile were taken using sterile syringes from sound gallbladders into sterile Bijou bottles & transported in ice to the laboratory. Stock dilutions were kept frozen, thawed only once then used or discarded.

**Sterilization;**
Glassware such as MacCarteny, Bijou and Universal bottles & flasks were sterilized in the autoclave at 15 pounds pressure for 15 minutes (121°C for 15 minutes). Petri dishes, tubes & glass beads were sterilized in the hot – air oven at 160°C for 90 minutes. Blood agar and brain-heart infusion broth were sterilized by autoclaving at 121°C for 15 minutes, whereas nutrient agar was sterilized at 115°C for 20 minutes.

**Incubation:**
All these tests were incubated at 37°C for 24~48 hours in dry-heat incubator.

**Culture media preparation:**
All media were dispensed under aseptic conditions in a laminar air flow cabinet type II (Prettl® Germany) provided with a fan, UV light lamp & flame. All media were obtained in a dehydrated form.

**Culture (culturing) technique:**
Discrete colony of the above strain was picked by means of standard wire loop & inoculated into brain-heart infusion broth; blood agar plates were flooded with the bacterial suspension into the brain-heart infusion broth, the flooding fluid was aspirated 10 mints later off the plates using sterile Pasteur pipettes with rubber teats. The plates were thereafter left to dry in the incubator for 10 mints.

**Experiments:**
The inhibitory affect of bile on the growth of the above mentioned organism was tested; according to the following procedures;

I. For each test organism a set of 4 test tubes was assigned, containing bile ‘as such’, bile with equal volume of normal saline, bile in 2 volumes of normal saline & bile in 4 volumes of normal saline

II. Using a standard loop, a loop-full amount of bacterial growth was inoculated into each of the 4 tubes & shaken until a homogenous suspension was established.

III. A blood agar plate was assigned for each of the test-organisms. The enumerated plates were divided into 4 quarters, enumerated 1~4, by a marker pen on the bottom surface.

IV. Using a standard loop, discrete colonies from the bacterial suspension 4 tubes were streaked onto the surface of the blood agar corresponding quarter.

V. Tubes & plates were incubated 24~48hrs.

**The bile drop experiment:**

I. A loop-full growth of each organism was inoculated into specified test tubes containing brain-heart- infusion broth, and shaken until a homogenous suspension is obtained.

II. Specified blood agar plates were flooded with the suspension, allowed to settle for 10 minutes thereafter the excess fluid aspirated using Pasteur pipettes with teats.

III. Plates were incubated for 10 minutes to dry in the dry heat incubator.

IV. A 50μl (1/20ml) drop of bile or one of its 3 successive dilutions was carefully dropped onto the middle of the specified enumerated plate quarter.

V. The plates were incubated for 24 – 48 hrs.

**RESULTS**
The MIC was 6.25 % of ox bile to *Staphylococcus albus, Corynebacterium pseudotuberculosis, Staphylococcus saprophyticus, and Micrococcus variant, 50% to E. coli, Pseudomonas spp. and Klebsiella spp., and 100 % (bile ‘as such’) to Staphylococcus albus, Escherichia vulneris, Staphylococcus aureus, Proteus spp. and Bacillus spp.* These results are summarized in table 1 and Figure 1.
Table1. The minimum inhibitory concentration (MIC) end points of bile of the ox against the growth of selected species of bacteria

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>(MIC) of ox bile (v/v)% of 0.85 normal saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus albus*</td>
<td>6.25</td>
</tr>
<tr>
<td>Corynebacterium pseudotuberculosis</td>
<td>6.25</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus*</td>
<td>6.25</td>
</tr>
<tr>
<td>Micrococcus variant*</td>
<td>6.25</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>50</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>50</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>50</td>
</tr>
<tr>
<td>Staphylococcus albus</td>
<td>100</td>
</tr>
<tr>
<td>Escherichia vulneris</td>
<td>100</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>100</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>100</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>100</td>
</tr>
</tbody>
</table>

* Microorganisms cultivated on blood agar after immersion in the specified ox bile concentration. (i.e. values represent the minimum bactericidal concentrations MBCs). Although Bacillus subtilis & Entrobacter spp were as well inhibited to varying extents, the inhibitions were not complete even at 100% bile concentration so by definition does not fit into the MIC.

Figure 1. Depicting the minimum inhibitory concentration (MIC) end points of bile of the ox on selected bacterial species

**DISCUSSION**

The present estimates, showing a graded response in bacterial sensitivity, suggest that the activities elicited by ox bile are bactericidal stated that the fact that fluoroquinolones act in a concentration-dependant manner rather than a time-dependant manner is evidence of their bactericidal activity. Andrews (2001)², stated that the minimum bactericidal concentration (MBC) is the lowest concentration of an antimicrobial that will prevent growth of an organism after subculture onto an antibiotic-free media. The statement is in agreement with the present determination of lower MIC values for the same organisms that were sub-cultured after immersion in bile (Staphylococcus albus, Staphylococcus saprophyticus and Micrococcus variant). Reeves et al., (1980)⁹, determining MIC values in 222 clinical isolates, also reported the observation that MIC estimates differ for the same antimicrobial agent
in the same organism when using different media or cultivation technique. The present minimum inhibitory concentration (MIC) estimates are in partial agreement with those estimates by Elsanousi et al., (2004)⁴, for deoxycholic acid against *Staph. aureus* & *E. coli*, and are different from their estimates for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, this is justified by the fact that deoxycholic acid constitutes but a small portion of whole bile, which suggests that additional antimicrobial activity is harbored & entertained in constituents of bile, including bile acids, other than deoxycholate.

**CONCLUSION**
The minimum inhibitory concentrations (MIC) of ox bile to these microorganisms were estimated and were smaller than those determined for bile salts in the same species estimated by other investigators. The graded MIC values are highly suggestive of a bactericidal activity.

**REFERENCES**

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