Studied aimed to explore the in vitro effect of ofloxacin and ornidazole (Mebatic) combination as compared with other drugs on aerobic and anaerobic bacteria. The study was done from March 2014 to March 2015 in the Department of Microbiology, Venus Medicine Research Centre, Baddi, Himachal Pradesh. A total of 1059 clinical samples were collected in a sterile container for aerobic and anaerobic culture. Identification of bacteria was done by microscopy and biochemical reaction. The antibiotic sensitivity testing was performed by cup plate method.

Out of 1059 samples, 605 samples showed the growth of bacteria. Further identification revealed the presence of nine different Gram negative and Gram positive organisms such as S. aureus, S. pneumoniae, C. trachomatis, N. gonorrhoeae Proteus spp., C. perfringens, Bacteroides spp., Yersinia spp. and Fusobacterium spp. The susceptibility of ofloxacin plus ornidazole (Mebatic) against S. aureus, S. pneumoniae, N. gonorrhoea, C. trachomatis, Proteus spp., Fusobacterium spp., C. perfringens, Bacteroides spp. and Yersinia spp. was > 85% which was high compared to metronidazole, clindamycin alone and, ceftriaxone plus metronidazole, levofloxacin plus metronidazole, moxifloxacin plus metronidazole in combinations. A high resistance to metronidazole (30-100%), clindamycin (25-92.6%), ceftriaxone plus metronidazole (39-88.4%), levofloxacin plus metronidazole (23-83.0%), ofloxacin plus metronidazole (16-67%) and moxifloxacin/metronidazole (18.9%-75.2%) was observed.

The study concluded that all the pathogens were found to be highly susceptible to ofloxacin/ornidazole compared to other antibiotics.

INTRODUCTION

The fluoroquinolone antibiotics are among the most widely used human medicine with a broad spectrum activity against most Gram-negative and Gram-positive bacteria. Quinolones rapidly inhibit deoxyribonucleic acid (DNA) synthesis by promoting cleavage of bacterial DNA in the DNA–enzyme complexes of DNA gyrase and type IV topoisomerase, resulting in rapid bacterial death (Hooper, 2000). Imidazole such as ornidazole and metronidazole is known to be quite active against Gram-negative anaerobes such as Bacteroides fragilis, Prevotella melaninogenica, Prevotella distens, Prevotella oralis, Prevotella intermedia and Fusobacterium spp. but does not appear to possess any clinically relevant activity against facultative anaerobes or obligate aerobes. The anaerobic gram-positive sporing bacilli such as Clostridium spp. are nearly always susceptible to imidazole (Neu 1991; Dollery 1999). Quinolones is active against aerobic and some anaerobic bacteria (King et al., 1982).

Antibiotic resistance among anaerobes has steadily increased since the early 1970s. Initially, antibiotic resistance among anaerobic bacteria went unnoticed for several reasons, predominantly because many mixed infections (David, 2004). The increase in antibiotic resistance among anaerobes has spawned intensive investigation into the mechanisms of resistance and resistance-gene transfer. The most frequently isolated antibiotic-resistant anaerobe is Bacteroides fragilis. Resistance is also seen among anaerobes that were previously considered to be highly susceptible to fluoroquinolone antibiotics, raising concerns about appropriate empirical therapy. The fluoroquinolones and nitroimidazoles have currently been using extensive worldwide for clinical applications because of their good bioavailability and pharmokinetic profile.

Ofloxacin is (the broad antibacterial spectrum of quinolones) having very high gram-negative activity, including moderate activity against Pseudomonas aeruginosa (Khan et al., 2008;
anu Chaudhary and Anurag Payasi

RESULTS AND DISCUSSION

A total of 1059 clinical samples of urine, blood, vaginal swab, dental abscess, intra-abdominal secretion and pus were collected from various hospitals across India and were processed for isolation of pathogenic bacteria. Out of the samples analyzed, 605 samples showed the growth (Table 1). Among the samples that showed the presence of pathogens, around 47.5% samples were of urine followed by blood (22.5%), vaginal swab (18.2%), dental abscess (5.8%), intra abdominal secretion (5.5%) and pus (1.3%) (Table 1).

MATERIAL AND METHODS

Bacterial isolates

The present study was carried out over a period of two years from March 2012 to March 2014 in the Department of Microbiology, Venus Medicine Research Centre, Baddi, Himachal Pradesh. A total of 1059 clinical samples consisting of blood, urine, vaginal swab, dental abscess, pus and intra-abdominal secretions were collected in a sterile container. On receipt of clinical samples, they were transferred into cooked meat media (Hi-Media, Mumbai, India) and cooked meat media were incubated for 40 to 48 hours at 35°C in an ambient atmosphere. After incubation, approximately 0.3 ml of the enrichment broth was aspirated and plated onto pre-reduced CDC anaerobic agar with 5% sheep blood (C plate), CDC anaerobic agar with 5% sheep blood and PEA, and CDC anaerobic agar with laked sheep blood, kanamycin and vancomycin (all media from BD, USA) for anaerobic culture. Then, all plates were incubated at 35 to 37 ºC in an anaerobic incubator (Thermo Forma, USA). A separate C plate was simultaneously inoculated, and incubated in 5% CO2 for 40 to 48 hours for growth comparison. Culture plates were removed after 40 to 48 hours incubation for inspection of growth, and re-incubated for a total of 6 days. Suspected growth of anaerobes was confirmed by aero-tolerance testing (Silva et al., 2003). For aerobic culture, subculture was done on blood agar, MacConkey agar and chocolate agar. Blood agar and Chocolate agar were incubated under 5-10% CO2. The growth of organisms was identified by colonial morphology, gram staining and biochemical tests as described earlier (Cheesbrough, 2000).

Antimicrobial susceptibility testing

Antibiotic susceptibility testing for metronidazole, clindamycin, ceftriaxone plus metronidazole, levofloxacin plus metronidazole, moxifloxacin plus metronidazole, and ofloxacin plus ornidazole (Mebatic) were determined with cup plate method as described by Bennet et al., (1966). Testing was performed using Brucella agar plates supplemented with 5 g of hemin and 1 g of vitamin K per millilitre plus 5% laked sheep blood. The plates were incubated for 48 h at 37 °C in anaerobic jars. The Bacteroides fragilis ATCC 25285 was used as a control.

Table 1A profile of clinical samples used as a source of the pathogenic isolates

<table>
<thead>
<tr>
<th>Clinical Samples</th>
<th>Total clinical samples</th>
<th>Samples showing growth</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>389</td>
<td>284</td>
<td>47.5</td>
</tr>
<tr>
<td>Blood</td>
<td>289</td>
<td>136</td>
<td>22.7</td>
</tr>
<tr>
<td>Vaginal Swab</td>
<td>176</td>
<td>109</td>
<td>18.2</td>
</tr>
<tr>
<td>Dental abscess</td>
<td>134</td>
<td>35</td>
<td>5.8</td>
</tr>
<tr>
<td>Intra-abdominal secretion</td>
<td>49</td>
<td>33</td>
<td>5.5</td>
</tr>
<tr>
<td>Pus</td>
<td>22</td>
<td>8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Figure 1 Profile of different clinical isolates isolated from various samples.

Figure 2 Prevalence of various pathogens.

Morphological and biochemical characterization of the samples revealed the presence of nine different Gram negative and Gram positive organisms that included S. aureus, S. pneumoniae, C. trachomatis, N. gonorrhoeae, Proteus spp., C. perfringens, Bacteroides spp., Yersinia spp. and Fusobacterium spp. The detailed profile of various organisms collected from various clinical samples is shown in Figure 1. Among the isolates, S. aureus (29.9%) was found to be the most dominant pathogen Figure 2. Several studies from India demonstrated the prevalence of S. aureus to be 34 to 54% in various clinical samples.
samples (Arora et al., 2010; Gopakrishnan and Suresh 2010; Mathews et al. 2010; Singh et al., 2013; Rezvan et al., 2009). A study performed by Rao et al. (2013) reported the prevalence of S. pneumoniae to be 27.8% which is similar to the current study where its prevalence rate was 20.1%. Jensen et al. (2003) reported 11.5% prevalence of C. trachomatis in his study, which is almost similar to our study (13%). Oknkow et al. (2014) showed that out of the total number of clinical samples, N. gonorrhoeae was detected in 15.2% of samples followed by Bacteriods spp. was (5.2%), and Yersinia spp, (0.67%) which are comparable to current investigation.  

Feglo et al. (2010) noted that C. perfringens was most prevalent pathogen (76.9%) in blood followed by Fusobacterium spp. (33.3%), Bacteroides spp. (31.2%), and Yersinia spp. (25%). Foulon et al. (2003) also showed that prevalence of Bacteroides spp. in blood samples was 26.2%, which is in accordance with the results of the present study. In vaginal swab samples prevalence of C. trachomatis was highest, accounting for 50.6%, whereas Bacteroides spp. contribute 62.5% in intra-abdominal secretions. In pus samples all the organisms contributed less significantly.

Antibiogram profile for all the pathogens isolated from various clinical samples is presented in Figure 3 and 4. In our study we found that the activity of ofloxacin plus ornidazole (Mebatic) was the highest among all the tested antibiotics. The susceptibility of ofloxacin plus ornidazole (Mebatic) against S. aureus, S. pneumoniae, N. gonorrhoea, C. trachomatis, Proteus spp., Fusobacterium spp., C. perfringens, Bacteroides spp. and Yersinia spp. was 91.7%, 92.6%, 95%, 97% 86.7%, 88.9%, 88.4%, 87.8%, and 92%, respectively which was high compared to metronidazole, clindamycin alone and, ceftriaxone plus metronidazole, levofloxacin plus metronidazole and moxifloxacin plus metronidazole in combinations.

The enhanced activity of Mebatic may be due to synergistic activity of ofloxacin and ornidazole. According to Bhardwaj et al. (2003) combination of a quinolone drug with a nitroimidazole drug enhances the antimicrobial properties against both aerobic and anaerobic bacterial infections. A high prevalence of resistance to metronidazole (30-100%), clindamycin (25-92.6%), ceftriaxone plus metronidazole (39-88.4%), levofloxacin plus metronidazole (23%-83.0%) and moxifloxacin/metronidazole (18.9%-75.2%) was observed.  

Dubruil et al. (1988) conducted a study on the 58 anaerobic strains (Bacteroides spp.) and observed that the combination of metronidazole plus ofloxacin had an additive bacteriostatic effect on 30 strains and a synergistic effect on 26 strains. No antagonism was noted with any strain.  

In the current investigation, S. aureus showed 92% resistance to clindamycin alone which is very high compared to previous study of Reddy et al. (2014) where they noticed that nearly 42.6% of S. aureus isolates were resistance to clindamycin. Recent data from Yardeni et al. (2013) reported that single daily dosing of ceftriaxone plus metronidazole is as safe and effective as the triple antibiotic regimen (ampicillin, gentamicin and metronidazole) and has significant advantages for the conservative therapy of complicated appendicitis in children. Similar experiments for levofloxacin plus metronidazole combination have been performed by Kelly et al. (2004) where they showed that levofloxacin (750 mg) and metronidazole (1,500 mg) may offer a more convenient once-daily treatment option for mixed aerobic-anaerobic infections, compared to the standard administration of metronidazole (500 mg every 8 h), however, the result of the current findings state that these combinations are developing high resistance which may be due to overuse.
Among all antibiotics tested, Mebatic revealed the highest activity against aerobic and anaerobic, Gram-negative and Gram-positive organisms. Further studies are needed to evaluate these combinations in clinical settings as they can play important role for the clinicians as viable alternative to fluoroquinolones in scenario of rising resistance to fluoroquinolones moieties.

CONCLUSION
As a result of decline in research on antibiotics and the problem of antibiotic resistance growing steadily, there is a need for newer and novel combinations of antibacterial agents. As the administration of ofloxacin and ornidazole has been reported to have favorable pharmacokinetic properties, the combination of ofloxacin and ornidazole has therefore, all the advantages needed for an effective antimicrobial therapy. Thus results of the present study concluded that the fixed dose combination of ofloxacin and ornidazole is an alternative and effective option for use in many mixed microbial infections occurred due to aerobic and anaerobic bacteria and pathogenic protozoa when compared to other antibiotic combinations, which might be due to resistance countering potential of Mebatic.

References
Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement. CLSI document M100- S23. 2013:Wayne, PA 19087 USA.


