STUDIES ON THE ANTIBIOTIC SENSITIVITY PATTERN OF ISOLATES OF P. MULTOCIDA FROM BUFFALOES


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ABSTRACT

Antibiotic susceptibility pattern of Pasteurella multocida causing hemorrhagic septicemia in buffaloes was determined. The Pasteurella multocida (n=11) were isolated from healthy (04) and diseased buffaloes (07) of four districts of Sargodha division. Isolates were identified by cultural, biochemical and serological characteristics, and confirmed by species specific polymerase chain reaction. All isolates were subjected to antibiotic susceptibility testing against gentamicin, erythromycin, amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, enrofloxacin, sulfadiazine and amikacin by disc diffusion method. Results revealed that P. multocida were highly sensitive to enrofloxacin (90.91 %) followed by gentamicin, chloramphenicol, ciprofloxacin and norfloxacin (72.73%). Susceptibility of P. multocida isolates was low against ampicillin and amoxicillin (45.45%), amikacin (36.36%), sulfadiazine (18.18%) and erythromycin (18.18%). It was concluded that quinolones (enrofloxacin, ciprofloxacin and norfloxacin) are better choice for the treatment of hemorrhagic septicemia as the level of resistance in P. multocida against the macrolides, sulfa drugs and beta lactams has increased enormously.

Key words: Hemorrhagic septicemia, Pasteurella multocida, Buffalo, Antibiotic susceptibility, Quinolones.

INTRODUCTION

Pasteurella multocida, a gram-negative bacterium, causes hemorrhagic septicemia (HS) which is an acute, often fatal disease of buffaloes and cattle. P. multocida is a natural inhabitant of the nasopharynx and occasional resident of intestines of healthy ruminants (Shivachandra et al., 2011). P. multocida are serologically classified as type A, B, C, D and E on the basis of capsular antigen. P. multocida serotype B: 2 is major cause of HS in Pakistan and has been involved in all outbreaks in Pakistan (Benkirane et al., 2002, Naz et al., 2012). The incidence of the disease is higher in buffaloes as compared to cattle (De Alwis et al., 1982, Hajikolaei et al., 2008; Khan et al., 2006). The disease process is triggered when host resistance is lowered due to various stress conditions (Naz et al., 2012).

P. multocida is a capsulated, non-motile, non-spore forming, gram negative coccobacilli. A number of serological techniques including agar gel precipitation and mouse protection test are used for confirmation of both vaccine and field isolates of P. multocida (Naz et al., 2012). Accuracy of serological identification of microbes is more reliable and specific in comparison with biochemical tests. The reliability of this identification system depends on antisera used, cross reactivity and differences in immune responsiveness of host animal used to raise serum (Munir et al., 2007).

HS is an acute disease making its treatment extremely difficult (Naz et al., 2012). Immediate and efficient treatment depends on the prior knowledge of recent antibiotic susceptibility patterns of causative agent. Although, antimicrobials have been widely used for the treatment of clinical infections, misuse and over use of antibiotics in clinical cases, veterinary and agricultural set ups pose a high risk for the selection of resistant bacteria (Nawaz et al., 2011). This has resulted in reduced efficacy of the antimicrobial agents that are currently available for the treatment of infections in animals. Emergence of multiple drug resistance and its transfer between microbes of different origins has compounded the problem to its maximum.

Different researchers have studied antibiotic resistance or sensitivity patterns in P. multocida isolated from different sources (Babetsa et al., 2012, Hendriksen et al., 2008, Huang et al., 2009, Kroemer et al., 2012, Markowska-Daniel et al., 2010, Mohamed et al., 2012, Shivachandra et al., 2011). Antibiotic resistance pattern of P. multocida has also been described in few of the previous studies from different area of Pakistan (Kalhor et al., 2010, Naz et al., 2012), but there are no reports on the antibiotic susceptibility pattern from the isolates from Sargodha Division of Punjab. Keeping this in mind the present study was designed to isolate, identify and determine the antibiotic susceptibility pattern of P. multocida isolates against antibiotics of different classes which are in use against HS.
MATERIALS AND METHODS

Samples: Blood samples from larynx/pharynx of slaughtered buffaloes (Healthy animals) and blood or tracheal swabs and long bones from dead or suspected animals (HS suspected) were collected from different areas of Sargodha division, Punjab. The samples were processed for isolation and identification of suspected etiological agents by standard methods (Carter 1984).

Isolation and identification: Samples were inoculated in Tryptic Soy Broth (TSB) and incubated at 37°C for 24 hours. Broth cultures were inoculated (multiple streaking) on tryptic soy agar (TSA) plate for purification of bacterial culture. The isolates were identified on the basis of colony characteristics, gram reaction, and biochemical characteristics using API (NE-20). The isolates were further confirmed serologically by agar gel precipitation test as described by (Heddleston et al., 1972) and mouse protection tests (Roberts 1947) and specie specific polymerase chain reaction (PCR) (Townsend et al., 2001).

In vitro antibiotic sensitivity of isolates: Antibiotic susceptibility pattern of P. multocida isolates against ten commercially available antibiotics (gentamicin, erythromycin, amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, enrofloxacin, sulfadiazine and amikacin) was determined by disc diffusion assay (Bauer et al.1966). The inoculums were prepared from fresh cultures of P. multocida, resuspended in phosphate buffer saline (0.5 McFarland). Inoculum was evenly spread on TSA plates with cotton swabs. Antibiotic discs were placed on inoculated agar plates and incubated at 37°C for 24 hours. Diameter of the zones of inhibition around each disc was measured and isolates were designated as sensitive, intermediate or resistant on the basis of zone of inhibition.

RESULTS AND DISCUSSION

The P. multocida (n=11) were isolated from diseased (07) and healthy buffaloes (04) from Sargodha division. Results of antibiotic sensitivity pattern of isolates are presented in table1.

Sensitivity of P. multocida isolates to enrofloxacin was highest (90.91 percent) followed by 72.73% to each of gentamicin, chloramphenicol, ciprofloxacin and norfloxacin. Sensitivity of isolates to ampicillin and amoxicillin was 45.45% while 36.36% isolate were sensitive to sulfadiazine. Lowest sensitivity was observed for to amikacin and erythromycin (18.18%).

High level of antibiotic susceptibility of P. multocida to enrofloxacin reported in this study is in agreement with previous studies by Aye et al., (2001), Anwar et al. (2000), Kromer et al. (2012), and Naz et al., (2012). In present study, ciprofloxacin and norfloxacin resistance was also observed in isolates of P. multocida which is in agreement with Carty et al. (2005). In contrast, Shayegh et al. (2009) and Naz et al., (2012) reported the absence of quinolones resistance in P. multocida. P. multocida were fairly sensitive to gentamicin (72.73%). Similar finding has also been observed by Balakrishnan et al., (2001), Verma et al., (2004) and Naz et al., (2012). Amikacin was one of the least effective antibiotics against P. multocida (18.18%). In contrast to our findings, Aye et al., 2001 found that most of the isolates of P. multocida were sensitive to amikacin. Susceptibility of P. multocida were moderate against beta lactam antibiotics (45.45%), which is higher than previously reported (25%) by Naz et al., (2012) and lower than Shikuma et al., (1985) and Wissing et al., (2001). P. multocida were generally resistant to sulfadiazine and erythromycin which is in agreement with Naz et al., (2012). Chloramphenicol, like quinolones, was also highly effective (72.73%) against P. multocida, which is in agreement with Balakrishnan et al., (2001) and Zahoor et al., (2006). In contrast, Naz et al., (2012) observed that 50% isolates of bovine origin were resistant to chloramphenicol.

Table 1: Antibiotic sensitivity pattern of P. multocida isolates against commonly used antibiotics

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Concentration</th>
<th>Sensitivity pattern, n (%)</th>
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<tbody>
<tr>
<td></td>
<td>µg/disk</td>
<td>S</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>5</td>
<td>10</td>
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<tr>
<td>Ciprofloxacin</td>
<td>10</td>
<td>8</td>
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<tr>
<td>Norfloxacin</td>
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<td>8</td>
</tr>
<tr>
<td>Erythromycin</td>
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<td>2</td>
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<tr>
<td>Sulfadiazine</td>
<td>10</td>
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</table>

S= Sensitive, R= Resistant, I= Intermediate.
Although isolates of *P. multocida* show resistance against many of the commonly used antibiotics, quinolones and chloramphenicol are still effective. It is insinuated that antibiotic susceptibility patterns *P. multocida* may be determined on larger scale for effective treatment of the HS.

**REFERENCES**


