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Antimicrobial Resistance in *Pasteurella multocida* Type B and *Mannheimia haemolytica* Isolates in Cattle and Buffaloes

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MARP, GISP, PSF designed the study. Authors NM, DMSBD, PSDA, MIW, NGNS performed sample collection and laboratory work. Authors MARP and GISP analyzed the results, wrote the protocol and wrote the first draft of the manuscript. Authors MARP and GISP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Hemorrhagic septicemia is a fatal septicemic disease in cattle and buffaloes in tropical countries. Young animals were more susceptible, and buffaloes were shown high percentage of mortality than cattle. *Mannheimia haemolytica* was described as an opportunistic pathogen in cattle and buffaloes and concurrent infection were also reported with primary other diseases. Only few studies were published in the country on these two bacterial organisms, information on antimicrobials resistance was limited in local herds. Lack of clinical breakpoints or host specific clinical breakpoints has been identified as a limiting factor to identify the phenotypic resistance. The objective of the study was to confirm the *Pasteurella multocida* isolates which received from clinical cases of hemorrhagic septicemia in the country. The second objectives were to determine phenotypic antimicrobial

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resistance in *Pasteurella multocida* clinical isolates and to compare with phenotypic antimicrobial; resistant phenotypes of *Mannheimia haemolytica.*
The clinical isolates of *Pasteurella multocida* were received (n=29) from the regional laboratories in the country from 2017-2019. *Manhenimia hemolytica* (n=49) was collected from our laboratory collection which were sampled from cattle and buffaloes from dry zone of the country previously. The resistance percentage of were *Pasteurella multocida* 72% and 29% for ciprofloxacin and cefotaxime. Resistance of cefotaxime was shown in *Pasteurella multocida* and none in *Manhenimia hemolytica.* Resistances to ciprofloxacin were shown high in *Pasteurella multocida and* low in *Manhenimia hemolytica.* Resistance to amoxicillin and clavulanic acid and tetracycline were found in both organisms. Phenotypic antimicrobial resistance was reported low in *Pasteurella multocida* and *Manhenimia hemolytica* in cattle and buffaloes**.** In conclusion, usage of sulfamethoxazole and quinolone group of antimicrobials for the pasteurellosis such as enrofloxacin, ciprofloxacin may have negative impact due to the high frequency of resistance observed.

Keywords: Pathogen; bacterial organisms; buffaloes; antimicrobials; Antimicrobial resistance.

1. INTRODUCTION

Hemorrhagic septicemia is a fatal septicemic disease in cattle and buffaloes [1,2,3]. It is an endemic disease in the dry zone of Sri Lanka, specially at the initial month of monsoon rainfalls [4]. Although two peaks of clinical raising were identified, year-round infection has also been reported [5]. Mostly, young animals around 12-24 months aged were reported common for the clinical infection [4,5]. Clinical incidence is common in buffaloes than cattle in the dry zone of the country (Figs. 1,2,3,4 in the supplementary document) [4]. Hemorrhagic septicemia in cattle and buffaloes is caused by *Pasteurella multocida*, two serotypes have been identified in Asia and Africa as B:2 and E:2 respectively [4,6]. *Pasteurella multocida* is Gram negative cocco bacilli and no growth is shown in MacConkey medium [7,8]. *Pasteurella multocida* has been recognized as the most common bacterial isolates found in bronchopneumonia in cattle [9,10]. In addition, *Pasteurella mutocida* and *Manhenimia hemolytica* are the most common respiratory pathogens found in claves [11]. Moreover, annual vaccination is carried out only in the endemic part of the country, both oil adjuvant and alum precipitated vaccines are used in annual vaccination and outbreak situation respectively [4]. Our laboratory is the national reference center for the identification of *Pasteurella multocida* in livestock, Sri Lanka. Therefore, all isolates bacterial cultured are received here for the confirmation as *Pasteurella multocida* B by Indirect haemagglutination inhibition test (IHA). No clinical breakpoint was described for *Manhenimia hemolytica,* and limited were described for *Pasteurella multocida*.

Mannheimia haemolytica was previously described as *Pasteurella haemolytica* and nomenclature was changed in 1999 [12,13]. It is a Gram negative, non-motile, coccobacilli and commonly found in the natural flora of mucous membrane in cattle and buffaloes [12,13]. Moreover, it is found in the upper respiratory tract of the ruminants such as cattle, sheep, and goats. *Mannheimia haemolytica* causes shipping fever in cattle and pneumonia and septicemia in sheep and goats [13]. Furthermore, *Mannheimia haemolytica* are grown on MacConkey medium. Mixed infections were reported common in buffaloes and cattle after the post stress situation.

Antimicrobial resistance is an emerging problem in veterinary medicine and both resistant phenotypes and genotypes have been identified in *Pasteurella multocida* clinical isolates [14]. Only a limited number of studies were published in the literature and practical difficulties and other unknown reason may cause difficulty in antimicrobial susceptibility testing. Lack of clinical breakpoints or host-specific clinical breakpoints has been identified as a limiting factor to identify the phenotypic resistance, genotypic evidence was identified in *Pasteurella multocida* such as *str*A, *tet*B, *cat* A III, sulII [14]. The study's first objective was to confirm the *Pasteurella multocida* isolates which received from clinical cases of hemorrhagic septicemia in the country. The second objectives were to determine phenotypic antimicrobial resistance in *Pasteurella multocida* and *Mannheimia haemolytica* clinical isolates and to compare among two species.

2. MATERIAL AND METHODS

The clinical isolates of *Pasteurella multocida* were received (n=29) from regional laboratories around the country from 2017-2019. *Manhenimia*

hemolytica (n=49) was collected from our laboratory collection which was sampled from cattle and buffaloes from dry zone of the country previously.

The isolates received from regional laboratories were grown in 5% sheep blood agar and colony morphology, biochemical tests were performed before the serological confirmation by IHA test. All isolates were used as antigens together with standards antiserum from *Pasteurella multocida* B:2 strain for the confirmation as described methods previously. Antimicrobial susceptibility testing was done according to methods described in EUCAST. The results were interpreted as described by EUCAST. Since no standard protocol was described for *M. hemolytica*, similar methods for *Pasteurella multocida* were used. Since no clinical breakpoint was found, results were not analyzed.

3. RESULTS

IHA were done for biochemically confirmed *Pasteurella multocida* isolates and *Manhenimia hemolytica* were identified by biochemical tests. In antimicrobial susceptibility testing, all *Pasteurella multocida* isolates were resistance for Sulfamethoxazole and no clear zone were observed. The summary of antimicrobial susceptibility testing was shown in Table 1 to 5.

4. DISCUSSION

Pasteurella multocida and *Manhenimia hemolytica* were found common respiratory system in cattle [15]. Since no clinical breakpoint was described for *Manhenimia hemolytica,* the diameters of the clear zone of the antimicrobial susceptibility testing were described as a separate table for each antimicrobial (www.eucast). Therefore, results can be interpreted in the future when clinical breakpoint was launched by EUCAST. The resistance percentage of were *Pasteurella multocida* 72% and 29% for ciprofloxacin and cefotaxime. Importantly, these two drugs are not used in bovine medicine and enrofloxacin is being used as only the quinolone group of drugs in Sri Lanka for treating clinical infection in cattle. A similar

Table 1. Antimicrobial resistance in *Pasteurella multocida* and *Manhenimia hemolytica* with published clinical breakpoints

Antimicrobial agent	Pasteurella multocida (n=29)		Manhenimia hemolytica (n=49)	
	Number of resistant isolates	% of Resistance	Number of resistant isolates	% of Resistance
cefotaxime	3	29	0	0
ciprofloxacin	21	72.41	3	6.12
Amoxicillin + Clavulanic acid	0	0	0	0
Tetracycline	0	0	0	0

Table 2. Disk diffusion summary of *Pasteurella multocida and* Manhenimia hemolytica *for* Amoxicillin without published clinical breakpoints

Diameter in mm for amoxicillin (AML 25)	Number of isolated in *Pasteurella multocida*	Number of isolates in *Manhenimia hemolytica*
10		1
20		
25	1	1
30	6	10
31	1	
32	4	6
33		1
34	5	5
35		1
36	5	3
37	1	
38	2	2
39		
40	4	19

Table 3. Disk diffusion summary of *Pasteurella multocida* and Manhenimia hemolytica for erythromycin

Diameter in mm for erythromycin (E 30)	Number of isolated in *Pasteurella multocida*	Number of isolates in Manhenimia hemolytica
0		8
18		1
21	1	3
22	1	2
24	3	3
25	6	2
26	6	5
27		1
28	2	7
29		1
30	8	14
36	2	
40		2

Table 4. Disk diffusion summary of *Pasteurella multocida* and *Manhenimia hemolytica* for streptomycin

Diameter in mm for streptomycin (S 25)	Number of isolated in *Pasteurella multocida*	Number of isolates in *Manhenimia hemolytica*
12	1	1
15		3
16	2	2
18	2	1
19	3	2
20	18	23
21		
24		
21		1
22	1	4
24	1	3
25		2
26	1	4
30		1
40		2

findings of the high percentage of resistance was observed in *Pasteurella multocida* in pigs by Yoon He-Oh et al. 2019 [16]. In contrast, recent study in Spain suggested low frequency of antimicrobial resistance except lincomycin in *Pasteurella multocida* [17]. In addition, no tetracycline resistances were shown both organisms in the study although oxytetracycline are being widely in bovine medicine in Sri Lanka. Similar finding showed in Iran by Khamesipour et al. [18] and no tetracycline resistance was observed for *Pasteurella multocida* in cattle [18]. In contrast, high frequency of resistance to oxytetracycline were observed by Timsit et al, 2017 [19]. Furthermore, antimicrobials such as amoxicillin, erythromycin, streptomycin, and chloramphenicol in which no clinical breakpoints were described diameter of clear zones were mentioned as descriptive information in this study (Table 4, Table 5). According to a study done by Tang et al, 2009, 93.1% of isolates were shown multi drug resistance in *Pateurella multocida* from pigs [20]. However, 27% *Pasteurella multocida* isolates were shown resistant to tetracycline in Japan [21]. 17.4% of *Pasteurella multocida* isolates from small ruminants were multi drug resistant in India [22]. Genetic evidence was observed for the number of antimicrobials such as beta-lactam antibiotics, tetracycline, aminoglycosides, sulfonamides, and chloramphenicol among different host range including cattle [23]. High percentage of resistance was reported for macrolids and lincosamide in a collection of

Table 5. Disk diffusion summary of *Pasteurella multocida* and *Manhenimia hemolytica* for chloramphenicol

Diameter in mm for chloramphenicol (C 30)	Number of isolated in *Pasteurella multocida*	Number of isolates in *Manhenimia hemolytica*
26		3
27		
28		3
29		
30	2	8
32	10	12
34	6	3
36	2	2
38		
40	9	16
24		
25		
26		
30		
40		

Pasteurella multocida from bovine and ovine host in France [24]. The high percentage of (97.2-99%*) flo*R were reported in caves which cause chloramphenicol resistance in host [25]. The high per-acute of beta lactam resistance were reported by *Manhenimia hemolitica* from cattle [26].

Trends of phenotypic resistance are the positive outcomes of this study. In addition, the clinical breakpoint of this study of *Pasteurella multocida* and *Manhenimia hemolytica* are from human and no separate clinical breakpoint are described for veterinary isolates by EUCAST. However, separate clinical breakpoint was described for limited veterinary isolates by the Clinical and laboratory standard Institute (CLSI).

Resistance of cefotaxime was shown in *Pasteurella multocida* and no isolates were resistant to cefotaxime in isolates of *Manhenimia hemolytica.* Resistances to ciprofloxacin were shown high in *Pasteurella multocida* and comparatively low in *Manhenimia hemolytica* (Table 3 to 5). However, resistance to amoxicillin and clavulanic acid and tetracycline in both organisms.

5. CONCLUSION

Phenotypic antimicrobial resistance was reported low in and antimicrobial is still effective for treating *Pasteurella multocida* and *Manhenimia hemolytica* in cattle and buffaloes. However, usage of sulfamethoxazole and quinolone group of antimicrobials such as enrofloxacin, ciprofloxacin for the pasteurellosis may have a negative impact due to the high frequency of resistance phenotypes observed. Currently using antimicrobials are still effective for treating respiratory infection in cattle and buffaloes.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study from Ethical Review committee at VRI in 2019.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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