RESEARCH ARTICLE

Phylogeny, Multiple Antibiotic Resistance Index and Biofilm Characterization of *Mannheimia* species Isolated from Clinical Cases of Pneumonia in Small Ruminants

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Abstract

Mannheimia is a Gram-negative, bipolar organism that is frequently involved in respiratory infections of bovines and small ruminants. In the present study, a total of 92 nasal swab samples were examined from enzootic pneumonia cases of sheep and goat, out of which 30 *Mannheimia* isolates have been identified. The 16S rRNA sequence analysis of the isolate revealed a close genetic distance between the *Mannheimia* species from different geographical regions. However, it formed a separate clade in the phylogenetic tree and the isolate is closely related to the *M. hemolytica* and *M. caviae* species. Three methods (Standard Tube method, Congo Red method and Microtiter Plate assay) were evaluated for identifying the biofilm forming *Mannheimia* isolates. Among them, Microtiter plate assay was the best method for quantification of biofilm production, by which 29 out of 30 isolates were identified as biofilm producers. In antibiogram study, the *Mannheimia* isolates exhibited resistance towards ampicillins, gentamicin, co-trimoxazole, tetracyclines and amoxicillin. All 30 isolates developed a multiple antibiotic resistance index (MAR) of more than 0.2. This indicates that there is a potential threat of antimicrobial resistance (AMR) transmission through food animals. In conclusion our study provides insights into the genetic relation between *Mannheimia* from India with other *Mannheimia* species from a different geographical region, and their capabilities of biofilm production.

Key words: Biofilms, *Mannheimia,* MAR-index, 16S rRNA, Phylogeny,

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Introduction

The *Mannheimia* species are one among the major pathogens of small ruminants that are involved in respiratory tract infections (Bkiri *et al*., 2021). The *Mannheimia* bacterium is a Gram negative, non-spore forming; non-motile bipolar/cocco-bacillary organisms that produces opportunistic infections in animals. These organisms, resides as commensals of the respiratory tracts and under stress conditions, converted to pathogenic *Mannheimia.* Several *Mannheimia* associated factors have been identified that promote the virulence and pathogenesis of the organism. The major virulence factors of *Mannheimia* include leukotoxin, OmpA, Capsule, metallo-endopetidase, siderophores, biofilm and cell adhesion (Gharibi *et al*., 2021).

Mannheimia has the ability to produce biofilm, an extracellular matrix composed of mucopolysaccharide, proteins and DNA, that encase the bacterial cells and offers a hostile environment for resistance against host immune defenses (Yilmaz *et al*., 2016). In apparently healthy animals, *Mannheimia* resides within the biofilms formed in the oropharynx and tonsillar crypts, whereas in clinical cases of pneumonia, the biofilms may exist in many parts of the nasal mucosa, nostrils, crypts of the trachea and respiratory tract (Pillai *et al.*, 2018). Within these biofilms the bacteria can be protected from therapeutic drugs, phagocytic cells and provide congenial environment for exchange of genetic elements between bacteria, thus acts as one of the potential sources of transfer of antibiotic resistance among the bacterial communities (Boukahil, 2016; Ocak and Turkyilmaz, 2022). In the present study, *Mannheimia* species were isolated from the clinical cases of pneumonia in sheep and goats from coastal districts of Andhra Pradesh state, India. The biofilm forming ability of the isolates, phylogenetic relationship,

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antibiogram and multiple antibiotic drug resistance indices was revealed through this study.

Materials And Methods

Sample Collection: A total of 92 nasal swab samples were collected from clinical cases of pneumonia in sheep and goats which exhibited clinical signs like nasal secretions, mucopurulent discharges from nostrils, coughing, sneezing, dull, depressed with high fever. The sterile swabs were inserted deep into the nasal cavity, rotated with pressure along the mucosa of the nasal concha and aseptically transported in sterile PBS to the laboratory at 4^0C for further processing (Abate and Fentie Kassa, 2023).

Primary Isolation of *Mannheimia*: The nasal swabs were enriched in Brain Heart Infusion broth (BHI) at 37^0C for 24 h, followed by inoculation onto BHI agar. The small pin-point dew drop like colonies on BHI agar were expanded in Blood agar (BA) and MacConkey agar (MCA). The grayish haemolytic colonies on BA and small pin-point pink colored colonies on MCA agar were maintained as pure cultures and stored at -80^0C by glycerol preservation method. At every step the bipolar/coccobacillary morphology was checked in Gram's staining. The pure cultures of *Mannheimia* isolates were biochemically characterized for Catalase, Oxidase, Indole, Methyl red, Voges-Proskauer, Triple sugar Iron (TSI) test and Urease test (Carter and Cole, 1990; Cruickshank, 1975)

Molecular Typing of *Mannheimia* Isolates: The pure cultures of the *Mannheimia* isolates were typed using primers targeting genus specific 16S rRNA gene of *Mannheimia*. The primers used for amplification were: Forward 5'- GCTAACTCCGTGCCAGCAG-3' and Reverse 5'-CGTGGACTACCAGGGTATCTAATC -3' which can amplify the target region with an amplicon size of 304 bp (Sahay *et al*.,2020). The DNA was extracted by boiling and snap chill method from the overnight grown pure cultures. The PCR reaction was optimized in 25µL reaction with 12.5 µL of Promega 2X master mix, 0.625 µL of forward primer, 0.625 µL of reverse primer, 1.25 µL of template DNA and 10 µL of nuclease free water. The PCR was standardized with thermal cyclic conditions of 94^0C for 5 min, 30 cycles of 94^0C for 30 sec, 56^0C for 60 sec, 72^0C for 1 min and final extension at 72^0C for 10 min. The amplified PCR products were analyzed in 1.5% agarose gels with 0.5 µg/ mL ethidium bromide. The PCR products were electrophoresed at 90V for 60 min in submarine gel electrophoresis unit (BIORAD, UK). The agarose gels were visualized under UV trans-illumination using BIORAD Gel documentation system, Syngene, UK. The PCR amplicon size was analyzed in comparison with that of the quantitative DNA ladder (Sisco Research Laboratories Pvt. Ltd, Mumbai).

Sequencing and Phylogenetic Analysis of *Mannheimia* Isolates

The purified DNA was sequenced by using two primers by Barcode Biosciences, Bengaluru. The sequences obtained were analyzed using Clustal omega, multiple sequence alignment (MSA) and phylogeny tool using MEGA 11 software. The overlapping sequences were aligned and constructed a *Mannheimia* genus specific partial length coding region of 16S rRNA. The details of the sequences retrieved from Genbank for MSA and phylogeny are M75080.1-*M. haemolytica* strain-USA, M75063.1-*M. haemolytica*–USA, NR_024899.1-*M. granulomatis*-Denmark, 24898.1-*M. ruminalis*-Denmark, 024896.1-*M. glucosida*–Denmark, NR_181249.1-*M. pernigra*–Switzerland, NR_179403.1-*M. massilioguelmaensis*, HM439607.1-*M. caviae*–Denmark, *M. haemolytica*-Canada.

Characterization of Biofilm Production by *Mannheimia*: The biofilm production of *Mannheimia* was characterized qualitatively by well established methods such as Standard Tube method (ST) described by Christensen *et al.* (1982), Congo Red Agar method (CRA) described by Freeman *et al.* (1989) and quantitatively by 96-well Microtiter plate (MTP) assay as described by Dhanawade *et al.* (2010).

Evaluation of Multiple Antibiotic Resistance Index (MAR): The MAR index of the *Mannheimia* isolates was phenotypically evaluated by Kirby-Bauer antibiotic disc diffusion method, with most commonly used antibiotics by field Veterinarians (Bauer *et al*., 1966). The results were analyzed and MAR index was calculated as the ratio of number of antibiotics to which the organism is resistant to the total no. of antibiotics to which the organism is exposed. A MAR index of ≥0.2 was considered as high-risk source of contamination where indiscriminate use of antibiotics was in practice.

Results and Discussion

In the present study, a total of 92 clinical cases of pneumonia in sheep and goats were studied for *Mannheimia* prevalence, its biofilm forming abilities, antibiotic drug resistance, MAR index and its phylogenetic analysis. Out of 92 clinical samples, 30 *Mannheimia* isolates were isolated by conventional methods. On Gram's staining, a clear coccobacillary/bipolar nature of the organisms was observed and biochemical characterization revealed that these isolates were positive for Catalase, Oxidase and negative for Indole, Methyl red, Voges-Proskauer, Triple sugar Iron (TSI) tests and Urease test which are characteristic for *Mannheimia*. On blood agar, these isolates developed small, greyish, rough, haemolytic colonies and on MCA, small, dew drop, pink pin-point colonies were observed.

Prevalence of *Mannheimia* in Clinical Cases of Pneumonia in Small Ruminants

A prevalence of 32.6% (30/92) of *Mannheimia* isolates was observed from the nasal swabs collected from clinical cases of pneumonic sheep and goats (Fig. 1). In general, most of the reports revealed a low prevalence rate of *Mannheimia* species isolated from nasal swabs, while a high prevalence of *Mannheimia* reported from the samples collected from the pneumonic lungs at post-mortem (Sahay *et al.*, 2020; Gharib *et al.*, 2021; Abate and Kassa, 2023). The type of sample, seasonal variations, sample processing techniques and many additional factors attributes for the perfect isolation of the *Mannheimia* species from the pneumonic sheep and goats. The nasal swabs collected from clinical cases are most useful for therapeutic studies and characterization of the organism during disease progression.

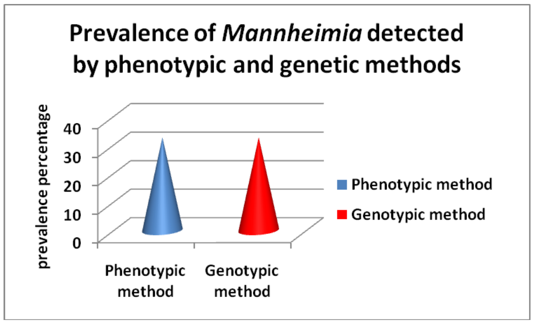

Fig. 1: Prevalence of *Mannheimia* detected by phenotypic and genetic methods

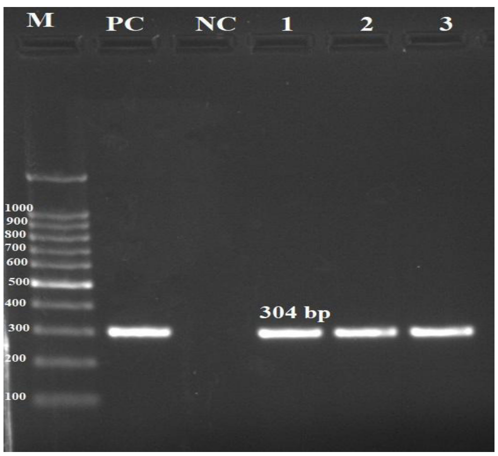

Fig 2: Detection of 16SrRNA gene of *Mannheimia* isolates (304 bp). M: 100 bp Ladder, PC: Positive Control (*Mannheimia* isolated from Sample Number S12); NC: Negative Control (*Pseudomonas aeruginosa*); Lane 1-3: Samples positive for *Mannheimia* species

Molecular Confirmation of *Mannheimia* Isolates

The morphologically and biochemically characterized *Mannheimia* isolates were subjected for confirmation in PCR targeting the gene coding for genus specific 16S rRNA. The genus specific primers amplified a product size of 304 bps using the template DNA of *Mannheimia* (Fig. 2). The results indicated that all 30 isolates were genetically confirmed the genera *Mannheimia*. The purified PCR product was sequenced and the overlapping sequences were aligned, constructed a partial sequence of 16S rRNA gene of *Mannheimia* using Multiple sequence alignment tool, Clustal Omega, EMBL Online program. The MSA revealed that the isolate belongs to the genus *Mannheimia*.

Phylogenetic Relationship of *Mannheimia* Isolate from Sheep with Those Available Sequences of *Mannheimia* species in Genbank

The genetic distances between *Mannheimia* isolated from Sheep, India and other *Mannheimia* strains from different geographical locations was evaluated in MEGA 11.0.13 software with maximum likelihood statistical method, and Tamura-Nei Model (Tamura *et al.*, 2021). The *Mannheimia* species isolated from sheep of Andhra Pradesh, India clustered into a different clade with close relation to *M. hemolytica*, Canada and *M. caviae*, Denmark. The remaining *Mannheimia* species clustered into different clades, and segregated species wise clusters, even though they are from different geographical regions. This implies a close genetic relation within the species and between the strains. The 16S rRNA is the most frequently used gene for confirming the taxa of a bacterium (Christensen and Bisgaard, 2010). Based on the phylogenetic analysis the isolated species may be closely related to *M. haemolytica* (Fig. 3) and it is a very useful tool for review of this family's taxa (Boudewijns, 2006).

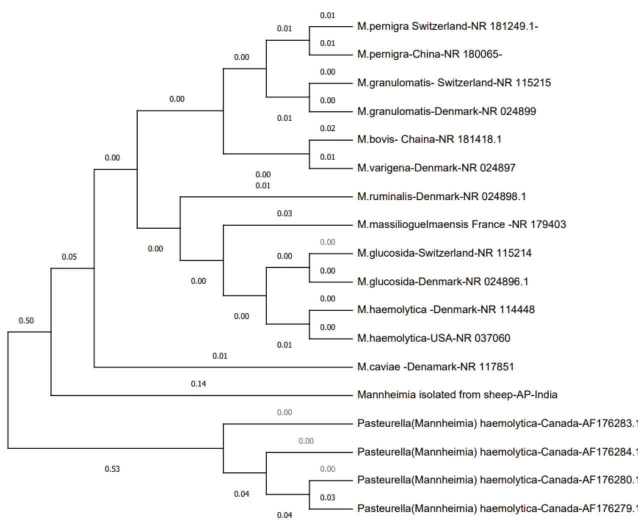

Fig. 3: Phylogenetic tree of *Mannheimia* isolated from India using partial 16S ribosomal RNA

Characterization of Biofilm Production by *Mannheimia* Isolates from Small Ruminants

The *Mannheimia* species have evolved through several ways of protection and escape from host immune system. One among the most common factor is biofilm formation in the respiratory air ways. In the present study, three methods were used to characterize the biofilm forming ability of *Mannheimia* isolates. Out of 30 *Mannheimia* isolates, the ST method detected 14 (46.66%) isolates as biofilm producers, CRA detected 25 (83.33%) isolates as biofilm producers and MTP assay detected and quantified 29 (96.66%) isolates as biofilm producers (Fig. 4). In ST method, out of 14 positives, 6 isolates were characterized as strong biofilm producers and 8 were moderate producers (Fig. 5). In CRA method, out of 25 positives, 12 were strong positives and 13 were moderate biofilm producers. In MTP assay, out of 29 positive isolates, 4 (13.33%) were strong, 16 (53.33%) were moderate, and 9 (30%) were categorized as weak biofilm producers. Among the three methods, the MTP assay was found to be the best, which can detect the production as well as quantification of biofilm formation by bacteria. The members of the *Pasteurellaceae* family, isolated from bovine respiratory infections can form biofilms within 4 h under static growth conditions (Tremblay *et al*., 2013). Under *in-vitro* conditions using bovine epithelial cells system or plastic surfaces, the biofilm was formed after 48 h of incubation at 37^{0}C (Boukahil *et al*., 2016). In our study the biofilm was recorded after 36 h of post incubation period in borosilicate glass test tubes & MTP assay while it took less than 18 h to develop black colonies on CRA agar. The possible reason for early detection of black colonies is that availability of adherent surface area in the form of CRA for the bacterium to form biofilms.

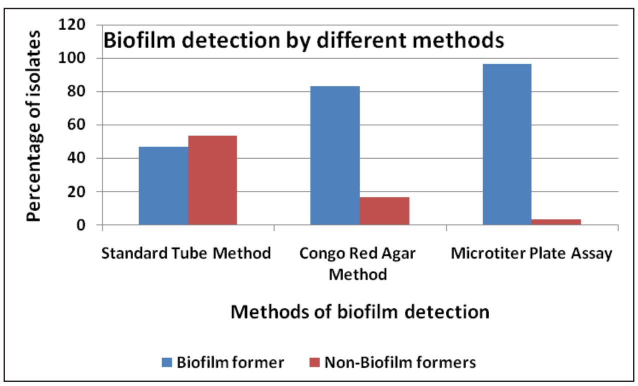

Fig. 4: Detection of Biofilm by different methods

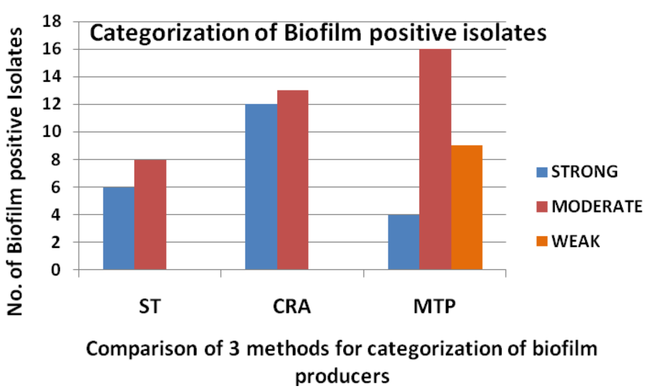

Fig 5: Comparison of methods for Categorization of Biofilm producing *Mannheimia*

Under stress conditions and underlying infections bring transient change from commensal bacterium to a respiratory pathogen. Several studies have proven that stress factors like epinephrine, nor-epinephrine and substance P can disperse the biofilm (Pillai *et al*., 2018). Similarly, the addition of mucin, a component of mucous, can reduce the formation of biofilm over bovine bronchial epithelial cells (Boukahil *et al*., 2016). The addition of 0.25% glucose to the medium was used in our study to enhance the production of biofilms by *Mannheimia* isolates. Most studies have focused on biofilm of *Mannheimia* isolates from bovine respiratory infections and the information on biofilm forming *Mannheimia* isolates from pneumonic cases of small ruminants is scanty. Through this paper, we reported the biofilm forming *Mannheimia*, isolated form enzootic pneumonia cases of sheep and goats. In addition to the protection from host immune system, the biofilms also confer resistance against certain antibiotic drugs (Olson *et al*., 2002, Boukahil *et al*., 2016; Sivarama *et al*., 2023).

Evaluation of Multiple Antibiotic Resistance (MAR) Index

The effective control of enzootic pneumonia in sheep and goats mainly rely on selection of appropriate chemotherapeutic agents. In field conditions, farmers and veterinarians depends on early diagnosis of the disease, and correct drug selection by antibiotic sensitivity test (ABST). In our study, for understanding drug resistance pattern of *Mannheimia* isolates, we have chosen the most commonly used antibiotics by the field vets, *viz*. Ampicillin, Gentamicin, Co-Trimoxazole, Tetracycline, Amoxicillin-Clavulanic acid, Ceftriaxone, Streptomycin, and Enrofloxacin. Among the 30 *Mannheimia*, the observed percent resistance pattern was Ampicillin (80%) followed by Gentamicin (50%), Co-Trimoxazole (43%), Tetracycline (40%), Amoxicillin-Clavulanic acid (40%), Ceftriaxone (36.6%), Streptomycin (36.6%), and Enrofloxacin (26.6%) (Fig. 6). An increase in antibiotic resistance of *Mannheimia* and *Pasteurella* species have been reported (Catry *et al*., 2006). Our study revealed that enrofloxacin, streptomycin and ceftriaxone are effective against most of the isolates. The results are in agreement with some reports and contradict with other reports. Seker *et al*. (2009) reported gentamicin as the most effective drug against *Mannheimia haemolytica* while Post *et al*. (1991) revealed 90% resistance to gentamicin. On the contrary, our study revealed that 50% isolates exhibit resistance to Gentamicin. Similar pattern of antibiogram was reported for *Mannheimia* isolated from pneumonic sheep, where it showed chloramphenicol and tetracyclines were the most effective while gentamicin was least effective drug (Marru *et al*., 2013). Similar to antibiogram results of our study, Ponnusamy *et al*. (2017) reported that the drugs enrofloxacin and ceftriaxone were the most effective drugs against *Mannheimia* isolates. The highest drug resistance towards pencillins followed by streptomycin, tetracyclines and gentamicin was reported by Sahay *et al*. (2020). All these results indicate a dissimilarity and confusion for a veterinarian to choose an effective therapeutic drug. Under these circumstances, the antibiotic susceptibility patterns need to be monitored and renewed periodically which will be useful for effective control of the disease (Onat *et al*., 2010).

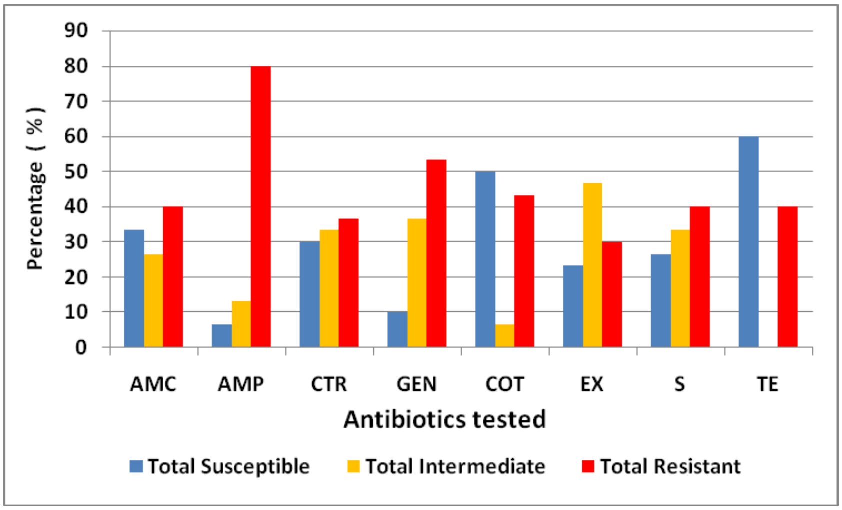

Fig. 6: Antibiogram of *Mannheimia* isolates

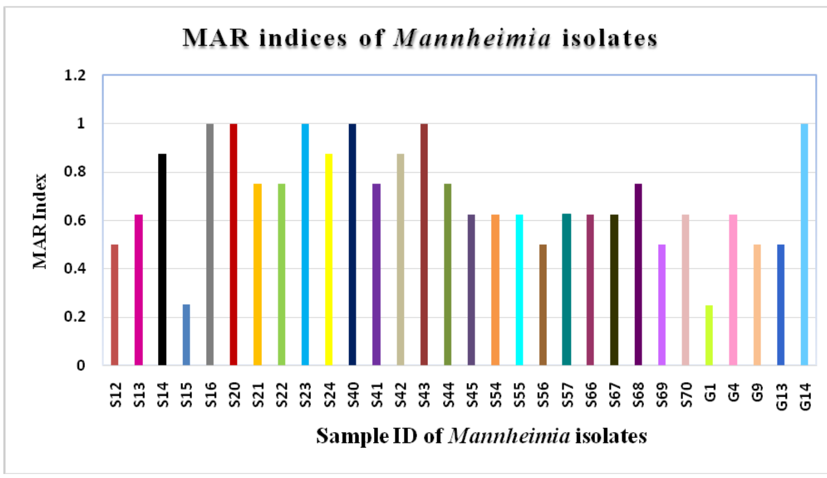

Fig. 7: MAR indices of *Mannheimia* isolates

The MAR index was calculated for each isolate and revealed a MAR index of greater than 0.2 for all isolates (Fig. 7). A MAR index of more than 0.2 indicates a potential threat to the public health from rising number of drug resistant bacteria entering a food chain. A MAR index average of 0.7 was reported in this study. Our findings are clinically significant because it provides data on emergence of multidrug resistant bacteria in least studied small ruminants. Additionally, the rising of biofilm forming strains of *Mannheimia* isolates further aggravates the emergence of MDR pathogens. Similar to our findings a high percentage of multidrug resistance was reported in >70% isolates of *Pasteurella* and *Mannheimia* from bovine respiratory infections of cattle (Depenbrock *et al*., 2021). In a study on *M. hamolytica* isolated from sheep in Vietnam revealed that more than 74% of the isolates were reported to be multidrug resistant (Van Nguyen *et al*., 2023). Tang *et al*. (2009) reported a gradual increase in antibiotic resistance from 47% in 2003 to 97.1% in 2007. The emergence of multidrug resistance bacteria was high in *Mannheimia* isolates in comparison to that of *Pasteurella* isolates (Singh *et al*., 2019). These findings alarm a potential threat of MDR *Mannheimia* pathogens entering the food chain of humans and animals, which needs to be addressed for effective control of multidrug resistant *Mannheimia*.

Conclusion

In conclusion, our study provides clinically significant data on prevalence of *Mannheimia* in clinical cases of pneumonia in sheep and goats with 32.6% prevalence in the study region. The isolate is closely related to the *M. haemolytica* species and segregated as a separate clade in the phylogenic tree. The biofilm forming ability of 29 *Mannheimia* isolates from sheep and goats were probably demonstrated for the first time by three different methods, and MTP assay is found to be the best method for biofilm quantification. *Mannheimia* isolates exhibit resistance towards ampicillins, gentamicin, co-trimoxazole, tetracyclines and amoxicillin and developed a MAR index of more than 0.2, which alarms a potential threat of emergence of multidrug resistant bacteria in food animals.

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References

Abate, F. M., & Fentie Kassa, T. (2023). Isolation and identification of *Mannheimia haemolytica* and *Pasteurella multocida* from symptomatic and asymptomatic sheep and their antibiotic susceptibility patterns in three selected districts of north Gondar zone, Gondar Ethiopia. *Veterinary Medicine and Science*, *9*(4), 1803–1811.

Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, *45*(4), 493–496.

Bkiri, D., Semmate, N., Boumart, Z., Safini, N., Fakri, F. Z., Bamouh, Z., Tadlaoui, K. O., Fellahi, S., Tligui, N., Fihri, O. F., & El Harrak, M. (2021). Biological and molecular characterization of a sheep pathogen isolate of *Mannheimia haemolytica* and leukotoxin production kinetics. *Veterinary World*, *14*(8), 2031–2040.

Boudewijns, M., Bakkers, J. M., Sturm, P. D., & Melchers, W. J. (2006). 16S rRNA gene sequencing and the routine clinical microbiology laboratory: a perfect marriage? *Journal of Clinical Microbiology*, *44*(9), 3469–3470.

Boukahil, I., & Czuprynski, C. J. (2016). *Mannheimia haemolytica* biofilm formation on bovine respiratory epithelial cells. *Veterinary Microbiology*, *197*, 129–136.

Cole, J. R. (Ed.). (1990). *Diagnostic Procedures in Veterinary Bacteriology and Mycology*. Academic Press.

Catry, B., Decostere, A., Schwarz, S., Kehrenberg, C., de Kruif, A., & Haesebrouck, F. (2006). Detection of tetracycline-resistant and susceptible pasteurellaceae in the nasopharynx of loose group-housed calves. *Veterinary Research Communications*, *30*(7), 707–715.

Christensen, G. D., Simpson, W. A., Bisno, A. L., & Beachey, E. H. (1982). Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infection and Immunity*, *37*(1), 318–326.

Christensen, H., & Bisgaard, M. (2010). Molecular classification and its impact on diagnostics and understanding the phylogeny and epidemiology of selected members of Pasteurellaceae of veterinary importance. *Berliner und Munchener Tierarztliche Wochenschrift*, *123*(1-2), 20–30.

Cruickshank, R., Duguid, J. P., Marmion, B. P., & Swain, R. H. A. (1975). *Medical Microbiology*, 12th Edit. Churchil livingstone Edinburgh, London and New York.

Depenbrock, S., Aly, S., Wenz, J., Williams, D., ElAshmawy, W., Clothier, K., Fritz, H., McArthur, G., Heller, M., & Chigerwe, M. (2021). *In-vitro* antibiotic resistance phenotypes of respiratory and enteric bacterial isolates from weaned dairy heifers in California. *PloS One*, *16*(11), e0260292.

Dhanawade, N. B., Kalorey, D. R., Srinivasan, R., Barbuddhe, S. B., & Kurkure, N. V. (2010). Detection of intercellular adhesion genes and biofilm production in *Staphylococcus aureus* isolated from bovine subclinical mastitis. *Veterinary Research Communications*, *34*(1), 81–89.

Freeman, D. J., Falkiner, F. R., & Keane, C. T. (1989). New method for detecting slime production by coagulase negative staphylococci. *Journal of Clinical Pathology*, *42*(8), 872–874.

Gharib Mombeni, E., Gharibi, D., Ghorbanpoor, M., Jabbari, A. R., & Cid, D. (2021). Molecular characterization of *Mannheimia haemolytica* associated with ovine and caprine pneumonic lung lesions. *Microbial Pathogenesis*, *153*, 104791.

Marru, H. D., Anijajo, T. T., & Hassen, A. A. (2013). A study on ovine pneumonic pasteurellosis: isolation and identification of Pasteurellae and their antibiogram susceptibility pattern in Haramaya District, Eastern Hararghe, Ethiopia. *BMC Veterinary Research*, *9*, 239.

Ocak, F., & Turkyilmaz, S. (2022). Investigation of antimicrobial resistance, biofilm production, biofilm associated virulence genes and integron genes of *pseudomonas aeroginosa* isolates obtained from animal clinical samples. *Israel Journal of Veterinary Medicine*, *77*(1), 15-26.

Olson, M. E., Ceri, H., Morck, D. W., Buret, A. G., & Read, R. R. (2002). Biofilm bacteria: formation and comparative susceptibility to antibiotics. *Canadian Journal of Veterinary Research 66*(2), 86–92.

Önat, K., Kahya, S., & Carli, K. T. (2010). Frequency and antibiotic susceptibility of *Pasteurella multocida* and *Mannheimia haemolytica* isolates from nasal cavities of cattle. *Turkish Journal of Veterinary & Animal Sciences*, *34*(1), 91-94.

Pillai, D. K., Cha, E., & Mosier, D. (2018). Role of the stress-associated chemicals norepinephrine, epinephrine and substance P in dispersal of *Mannheimia haemolytica* from biofilms. *Veterinary Microbiology*, *215*, 11–17.

Ponnusamy, P., Masilamoni, B. S., Ranjith, K. M., & Manickam, R. (2017). Isolation, identification and antibiogram of *Mannheimia hemolytica* associated with caprine pneumonia in the Cauvery Delta Region of Tamil Nadu, India. *International Journal of Current Microbiology and Applied Sciences, 6*(9), 3118-3122.

Post, K. W., Cole, N. A., & Raleigh, R. H. (1991). In vitro antimicrobial susceptibility of Pasteurella haemolytica and Pasteurella multocida recovered from cattle with bovine respiratory disease complex. *Journal of Veterinary Diagnostic Investigation Inc*, *3*(2), 124–126.

Sahay, S., Natesan, K., Prajapati, A., Kalleshmurthy, T., Shome, B. R., Rahman, H., & Shome, R. (2020). Prevalence and antibiotic susceptibility of *Mannheimia haemolytica* and *Pasteurella multocida* isolated from ovine respiratory infection: A study from Karnataka, Southern India. *Veterinary World*, *13*(9), 1947–1954.

Seker, E., Kuyucuoglu, Y., & Konak, S. (2009). Bacterial examinations in the nasal cavity of apparently healthy and unhealthy Holstein cattle. *Journal of Animal and Veterinary Advances*, *8*(11), 2355-2359.

Singh, F., Sonawane, G. G., & Meena, R. K. (2019). Pathology, isolation and characterisation of virulent and diverse *Mannheimia haemolytica* and *Pasteurella multocida* associated with fatal pneumonia in sheep, Rajasthan, India. *Comparative Clinical Pathology*, *28*, 531-540.

Sivarama Krishna Gollapalli, S. C. V., & Lokeshwari, R. (2023). The interaction between the antimicrobial compounds and the biofilms of Staphylococcus species isolated from bovine mastitis. *The Pharma Innovation 12(5),* 1933-1939.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027.

Tang, X., Zhao, Z., Hu, J., Wu, B., Cai, X., He, Q., & Chen, H. (2009). Isolation, antimicrobial resistance, and virulence genes of *Pasteurella multocida* strains from swine in China. *Journal of Clinical Microbiology*, *47*(4), 951–958.

Tremblay, Y. D., Deslandes, V., & Jacques, M. (2013). *Actinobacillus pleuropneumoniae* genes expression in biofilms cultured under static conditions and in a drip-flow apparatus. *BMC Genomics*, *14*, 364.

Van Nguyen, P., Le, C. T., Ho, X. T. T., Truong, P. H., Van Loi, B., & Nguyen, K. C. T. (2023). First report of antimicrobial resistance of *Mannheimia haemolytica* from Phan Rang sheep in Vietnam. *Pakistan Veterinary Journal*, *48(1),* 41-48.

Yılmaz, E. S., & Güvensen, N. C. (2016). *In vitro* biofilm formation in ESBL producing *Escherichia coli* isolates from cage birds. *Asian Pacific Journal of Tropical Medicine*, *9*(11), 1069–1074.