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Descriptive genomic analysis of antibiotic resistance in *Pasteurella multocida* isolates from India

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Abstract

Antibiotic resistance in Pasteurella multocida (P. multocida) poses a significant threat to both animal and public health. This study investigates the genomic mechanisms underlying antibiotic resistance in P. multocida strains isolated from various hosts in India, including buffalo, cattle, sheep, and pigs, as well as other species such as ducks and deer. A total of 40 whole genome sequences of *P. multocida* strains were analyzed, revealing that 17.5% harbored resistance genes, with notable resistance to antibiotics such as streptomycin, sulfamethoxazole, and trimethoprim. The ResFinder tool and Comprehensive Antibiotic Resistance Database (CARD) were employed to identify resistance genes. The presence of integrative and conjugative elements (ICEs) was detected using the ICEfinder tool, while MobileElementFinder was used to identify mobile genetic elements associated with resistance genes. Origins of transfer (oriT) were determined using oriTfinder, and efflux pumps were identified using resources from the Bacterial and Viral Bioinformatics Resource Center. Prophage sequences were annotated using PHASTEST. The study highlights the significant role of efflux pumps and oriT in the survival of resistant strains. The IncQ1 plasmid, identified in buffalo-derived strains such as PmBUFF2016HRY and ABT/RAWAL/2015/HSR, indicates a potential for multi-drug resistance spread across bacterial populations. Additionally, sheep-derived strains exhibited a high prevalence of prophages and genetic elements like integrase and transposase, making them key reservoirs for resistance evolution. This work provides valuable insights into the genomic mechanisms driving antibiotic resistance in P. multocida, offering a foundation for developing targeted strategies to curb resistance spread.

Introduction

Pasteurella multocida (P. multocida), is a pathogenic bacterium of significant concern in veterinary medicine due to its role in causing a range of diseases in livestock, including

hemorrhagic septicemia, pneumonia, and respiratory infections (1-3). In India, this bacterium is prevalent across various animal species, including buffalo, cattle, sheep, and pigs, leading to substantial economic losses in the agricultural

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sector (4). The zoonotic potential of *P. multocida* also raises public health concerns, particularly in regions where close human-animal interactions occur (5).

The increasing prevalence of antibiotic-resistant *P. multocida* strains has emerged as a critical issue, complicating the treatment of infections and increasing the risk of resistant strains spreading within animal populations (6). Resistance to commonly used antibiotics such as ampicillin, tetracycline, and streptomycin has been reported in various regions of India, necessitating a closer examination of the underlying resistance mechanisms (7).

Antibiotic resistance is often driven by the presence of mobile genetic elements and integrative and conjugative elements (ICEs), which facilitate the horizontal transfer of resistance genes among bacteria (8). These genetic components, coupled with efflux pumps and other resistance mechanisms, contribute to the persistence and spread of antibiotic resistance in *P. multocida* (9). Understanding these mechanisms is essential not only for managing current infections but also for predicting and preventing future outbreaks of resistant strains.

To address this issue, molecular techniques and whole-genome sequencing have become essential tools for identifying *P. multocida* and characterizing its resistance profiles. These methods allow for the detection of resistance genes, mobile genetic elements, and integrative and conjugative elements (ICEs), which play a pivotal role in the dissemination of resistance (10). Such insights are crucial for developing targeted interventions to manage and mitigate the impact of antibiotic-resistant *P. multocida* strains.

The primary objective of this study is to investigate the genomic mechanisms underlying antibiotic resistance in *P. multocida* strains isolated from various animal hosts in India. By analyzing resistance genes, mobile genetic elements, and other genomic features, this study aims to contribute to the understanding of resistance

patterns in *P. multocida* and inform strategies for effective management and control.

Material and Methods

Publicly available *P. multocida* whole-genome sequences of 40 isolates from various hosts in India, collected between 2000 and 2020, were obtained from the National Center for Biotechnology Information (NCBI) (11) and the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) (12).

The antibiotic resistance genes in the *P. multocida* strains were identified using the ResFinder tool (13-15) and the Comprehensive Antibiotic Resistance Database (CARD) (16). Additionally, integrative and conjugative elements (ICEs) were detected using the ICEfinder tool (17), with a focus on T4SStype ICEs, AICEs, and IMEs. Mobile genetic elements and their association with resistance genes were identified using MobileElementFinder (18), while the origins of transfer (oriT) were determined using oriTfinder (19). Prophage sequences were identified, annotated, and visualized using PHASTEST (20-22), and efflux pumps within the bacterial genomes were identified using resources from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) (12). The Stacked bar charts of the prophages were created using Data wrapper.

Results

Identification of Resistance Genes

The antibiotic resistance analysis of 40 bacterial whole genome sequences using the ResFinder tool and The Comprehensive Antibiotic Resistance Database (CARD) revealed significant insights into the resistance patterns of P. multocida strains. Among the whole genome sequences analyzed, 7 including the P. multocida strains PVNRTVU1, SDHB, CUL-TANUVAS-2020, PmBUFF2016HRY. ABT/RAWAL/2015/HSR. Anand1_buffalo, and HS SKN01 (NCBI IDs SAMN21524632, SAMN15406116, SAMN26179620, SAMN25692016,

SAMN05603852 respectively) were found to harbor resistance genes, accounting for 17.5% of the total strains, with 6 of these strains originating from *Bubalus bubalis* (buffalo). Notably, 15% of the *P. multocida* strains exhibited resistance to streptomycin, indicating a widespread resistance mechanism against this antibiotic. Additionally,

resistance to sulfamethoxazole and trimethoprim was observed in 15% and 10% of the bacterial genomes, respectively. Resistance to doxycycline and tetracycline was observed in 12.5% of the bacterial genomes each, while resistance to chloramphenicol and florfenicol was found in 12.5% and 7.5% of the genomes, respectively, the results are further depicted in Table 1.

Table 1. Percentage of antibiotic resistance in *P. multocida* strains used in this study

Sl. No.	Antibiotic	Percentage of Resistance (%)
1	Streptomycin	15
2	Kanamycin	15
3	Sulfamethoxazole	15
4	Doxycycline	12.5
5	Tetracycline	12.5
6	Chloramphenicol	12.5
7	Neomycin	10
8	Trimethoprim	10
9	Florfenicol	7.5
10	Unknown Aminoglycoside	5
11	Lividomycin	5
12	Paromomycin	5
13	Ribostamycin	5
14	Amoxicillin	5
15	Ampicillin	5
16	Cephalothin	5
17	Piperacillin	5
18	Ticarcillin	5
19	Minocycline	5

The study further revealed that the P. multocida PmBUFF2016HRY harbors multiple resistance genes, raising concerns for future treatment strategies. This strain possesses the catA2 gene, which confers resistance to chloramphenicol, and the blaTEM-1B gene, which provides resistance to a range of β -lactam antibiotics such as amoxicillin. ampicillin, piperacillin. and Additionally, the aph (3')-Ia gene contributes to resistance against several aminoglycosides. Similarly, the *P. multocida* subsp. multocida strain ABT/RAWAL/2015/HSR also carries the catA2 and blaTEM-1B genes, resulting in resistance to both chloramphenicol and β-lactam antibiotics. The study also reveals that SNP mutations in most of the strains could lead to future mutations and resistance. Specifically, mutations such as R234F, D350N, and S357N could result in resistance to elfamycin, cephalosporin, cephamycin, and penam, respectively.

Identification of integrative and conjugative elements (ICE)

Out of 40 bacterial whole genome sequences analyzed using the ICEfinder tool, 8 sequences of *P. multocida* strains including NIVEDIPm31, NIVEDIPm1, HS SKN01, CUL-TANUVAS-2020, PMS 19, PVNRTVU1, SDHB, and Anand1_buffalo (NCBI IDs SAMN29723026,

SAMN29723022. SAMN05603852. SAMN26179620, SAMN16691848. SAMN21524632, SAMN15406116, and SAMN0246963 respectively) were found to harbor an Integrative and Conjugative Element (ICE), accounting for 20% of the total strains. These P. multocida strains provided significant insights into the presence and characteristics of ICEs and Integrative and Mobilizable Elements (IMEs) within their genomes. The presence of key components such as integrase, relaxase, and various Type IV Secretion System (T4SS) elements, including PrgJ, T4CP, and others, in strains like NIVEDIPm31 and NIVEDIPm1, highlights their potential for horizontal gene transfer. This capability is crucial for the spread of genetic material, including antibiotic resistance genes, across bacterial populations. The P. multocida HS SKN01 strain, with its extensive array of T4SS components, and the CUL-TANUVAS 2020 strain, with unique elements like Orf169_F from Shewanella sp., underscore the adaptability of these strains to various environmental conditions, possibly enhancing their resistance to antibiotics. The detection of ICEs in strains such as PMS 19, PVNRTVU1, SDHB, and Anand1 buffalo further emphasizes the potential for genetic exchange and the acquisition of resistance traits. These strains possess various combinations of integrase, relaxase, T4CP, and other T4SS components, which facilitate the integration and mobilization of genetic elements. Notably, the presence of Orf14_Tn and Orf169_F in some strains indicates a robust mechanism for genetic adaptation, enhancing their survival in diverse environments. The observation that most strains harboring antibiotic resistance genes also contain ICEs suggests a strong correlation between these elements and resistance, pointing to the critical role of ICEs in the dissemination of resistance genes among bacterial populations.

Identification of mobile genetic elements

The identification of mobile genetic elements (MGEs) and their relationship to antimicrobial resistance genes and virulence factors in the bacterial genomes, analysed using MobileElementFinder, revealed that Р. the multocida strains HS SKN01, PmBUFF2016HRY, ABT/RAWAL/2015/HSR. SDHB. and Anand1_buffalo (NCBI IDs SAMN05603852, SAMN25692016, SAMN15457914, SAMN15406116 and SAMN02469637 respectively) possess MGEs. In the strains PmBUFF2016HRY, ABT/RAWAL/2015/HSR, and Anand1 buffalo, the identified MGEs are all insertion sequences, including ISAeca1 (IS30), ISKpn13 (IS5), IS26 (IS160, IS26L, IS26R, IS6, IS140, IS46), and ISVsa3 (ISVs3). However, these MGEs do not confer resistance to any drugs. In contrast, in the P. multocida strain HS SKN01, the MGE ISVsa3 is responsible for transferring the tet (A) and floR genes, which confer resistance to doxycycline, tetracycline, florfenicol. and chloramphenicol. Further analysis highlighted that the P. multocida strains PmBUFF2016HRY and ABT/RAWAL/2015/HSR contain the plasmid IncO1, which carries the blaTEM-1B, sul2, aph (6)-Id, and aph(3")-Ib genes. These genes confer resistance to antibiotics such as cephalothin, piperacillin, ticarcillin, amoxicillin, ampicillin, sulfamethoxazole, and streptomycin. Additionally, the P. multocida strain SDHB contains the MGEassociated floR gene, which provides resistance to chloramphenicol and florfenicol.

Identification of origin of transfers in DNA sequences

OriTfinder identified strains PVNRTVU1, SDHB, CUL-TANUVAS 2020, and HS SKN01 (NCBI IDs SAMN21524632, SAMN15406116, SAMN26179620, and SAMN05603852 respectively) as having origins of transfer (oriT) in their genomes, each with a length of 107 bp. In contrast, no oriT was predicted in strains PmBUFF2016 HRY and ABT/RAWAL/2015/HSR (NCBI IDs SAMN25692016, and SAMN15457914 respectively); however, Putative Relaxase and

intergenic sequences flanking the relaxase gene were present.

Identification of prophage

The analysis of *P. multocida* bacterial genomes using the PHASTEST tool revealed significant differences in the presence of prophages. Out of the 40 whole bacterial genomes analyzed, 38 contained at least one prophage region. The strain NIVEDI/PMS-1 showed the highest number of prophage regions, followed by NIVEDIPm31, NIVEDIPm22, NIVEDIPm9, NIVEDIPm1, PMS 14. and NIVEDIPm10 (NCBI IDs SAMN05717776. SAMN29723026. SAMN29723024. SAMN29723016. SAMN16691847, SAMN29722877, and SAMN29723017 respectively). The strain HS SKN01 is the only multidrug-resistant strain that possesses all the integrase, holin, transposases, and terminase. The genome map of HS SKN01 is shown in Figure 1. In contrast, the strains Anand1_buffalo and Anand1_cattle did not contain any prophages. The stacked bar chart showing the prophages present in the present P. multocida strains is depicted in Figure 2.

Identification of Efflux Pumps

The Bacterial and Viral Bioinformatics Resource Center (BV-BRC) analysis highlights the larger number of efflux pumps present in the *P. multocida* strains HS SKN01, Anand1_buffalo, and SDHB (NCBI IDs SAMN05603852, SAMN02469637, and SAMN15406116 respectively). Furthermore, the antibiotic-resistant strains identified using the ResFinder tool also possess a higher number of efflux pumps, further emphasizing the role of efflux pumps in conferring resistance to bacterial strains. The data on the efflux pumps present in each strain are listed in Table 2.

Discussion

The analysis of 40 whole genome sequences of the *P. multocida* strains revealed a concerning prevalence of antibiotic resistance, with 17.5% of the isolates harboring resistance genes. This finding

underscores the challenge of treating *P. multocida* infections in India, as these resistant strains can withstand commonly used antibiotics, posing significant public health risks. A notable 6 out of 7 antibiotic-resistant strains showed resistance to streptomycin, indicating a widespread mechanism of resistance to this antibiotic. Additionally, resistance to sulfamethoxazole and trimethoprim was detected in 15% and 10% of the isolates, respectively, highlighting the growing resistance to these commonly used antibiotics.

The presence of multiple resistance genes in certain multocida isolates. such as ABT/RAWAL/2015/HSR strain. complicates treatment strategies due to their multifaceted resistance mechanisms. The identification of Integrative and Conjugative Elements (ICEs) in strains like NIVEDIPm31 and NIVEDIPm1 indicates a significant potential for genetic exchange, a key driver in the spread of antibiotic resistance. While these strains do not currently harbor resistance genes, the presence of ICEs suggests a latent threat of resistance gene acquisition.

The complexity of ICE-related components in resistant strains such as HS SKN01, CUL-TANUVAS 2020, and SDHB demonstrates their capability for genetic exchange, potentially facilitating the spread of resistance genes. The presence of mobile genetic elements (MGEs) in certain strains, such as HS SKN01 and SDHB. further emphasizes the need for focused monitoring and control measures to prevent the dissemination of resistance. The identification of the IncQ1 plasmid in strains PmBUFF2016HRY ABT/RAWAL/2015/HSR highlights the role of mobile genetic elements in facilitating multidrug resistance, complicating treatment strategies and posing a significant public health challenge. The identification of origins of transfer (oriT) in specific strains suggests their capability for horizontal gene transfer, contributing to the spread of resistance.

Sequence length: 2.446,354 bps Number of phage regions found: 4 Total number of genes found: 2,115 Number of phage genes found: 114 Number of bacterial genes found: 2,001

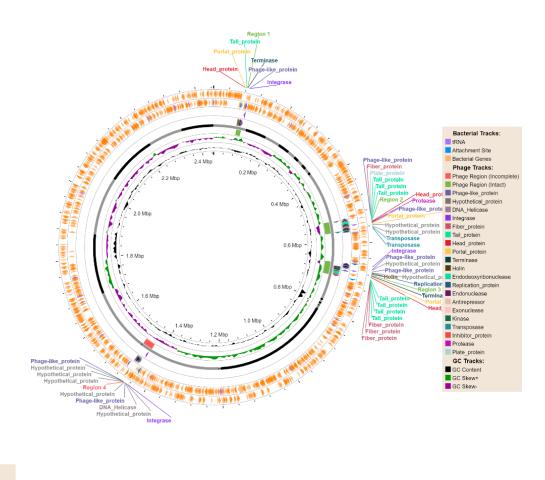


Fig. 1. Genome map of the *P. multocida* strain HS SKN01, generated using PHASTEST.

Interestingly, strains isolated from sheep hosts exhibited the highest number of prophage regions, which may increase genomic stress and drive bacterial evolution. The elevated presence of genes associated with genetic exchange in these strains suggests they may be particularly prone to acquiring and spreading resistance genes.

Overall, the study underscores the complexity of resistance mechanisms in *P. multocida* strains and the critical role of mobile genetic elements, ICEs, and efflux pumps in antibiotic resistance. Continued surveillance and targeted interventions are essential to mitigate the risk of widespread antibiotic resistance in *P. multocida* populations.

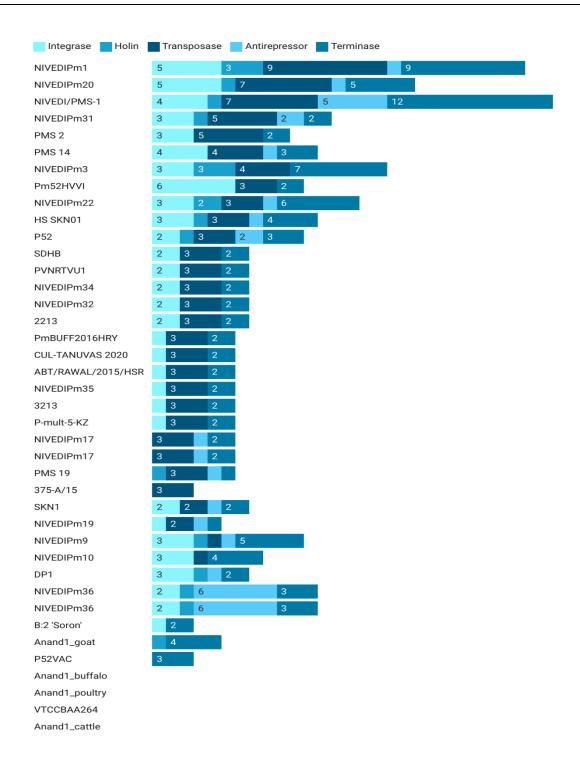


Fig. 2. Stacked bar chart showing the prophages present in the *P. multocida* strains, further highlighting Integrase, Holin, Transposases, Antirepressors, and Terminase

Table 2. Number of Efflux pumps present in the <i>P. multocida</i> strains used in this study	Table 2	2. Number	of Efflux	pumps p	oresent in t	he <i>P</i> .	multocida	strains	used in t	his study
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Genome Name	No of pumps	Genome Name	No of pumps
HS SKN01	13	2213	4
Anand1_buffalo	12	3213	4
SDHB	11	B:2 'Soron'	4
ABT/RAWAL/2015/HS	11	DP1	4
PmBUFF2016HRY	10	P52	4
Anand1_poultry	9	PMS 14	4
PVNRTVU1	8	PMS 19	4
NIVEDI/PMS-1	6	PMS 2	4
Anand1_goat	5	SKN1	4
P52VAC	5	NIVEDIPm17	4
VTCCBAA264	5	NIVEDIPm20	4
CUL-TANUVAS 2020	4	NIVEDIPm22	4
NIVEDIPm1	4	NIVEDIPm31	4
NIVEDIPm10	4	NIVEDIPm32	4
NIVEDIPm19	4	NIVEDIPm34	4
NIVEDIPm3	4	NIVEDIPm35	4
NIVEDIPm9	4	NIVEDIPm36	4
PMS 14	4	Pm52HVVI	4
PMS 19	4	375-A/15	2
PMS 2	4	Anand1_cattle	2

Conclusion

This study provides critical insights into the antibiotic resistance mechanisms in P. multocida strains from India, revealing that 17.5% of the isolates harbor resistance genes, with significant resistance to key antibiotics. The identification of multiple resistance genes and Integrative and Conjugative Elements (ICEs) underscores the role of horizontal gene transfer in the spread of resistance. Sheep-derived strains, with their high number of prophage regions and genetic elements, and buffalo-derived strains, harboring mobile genetic elements like the IncQ1 plasmid, present particular challenges due to their potential to spread multidrug resistance. These findings highlight the need for continued surveillance and targeted interventions to mitigate the spread of antibiotic resistance in *P. multocida*, especially in strains from sheep and buffalo. Focused efforts are essential to protect the efficacy of current treatments and safeguard public and animal health in India.

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Ethical approval

Not applicable.

Conflict of interest statement

There is no conflict of interest.

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