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Determination of Antimicrobial Activity of *Pseudomonas aeruginosa* Isolated from Dorper Sheep Milk with Sub-clinical Mastitis Infection

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ABSTRACT

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* has emerged as a major health issue to the world's small ruminant dairy industry. Antimicrobial susceptibility testing (AST) is crucial for identifying resistant *P. aeruginosa* strains linked to milk-borne mastitis infections in Dorper sheep at the farm level in order to create efficient management measures. AST tests of Dorper sheep isolates revealed that isolate 46-1 was resistant to all tested antibiotics, with intermediate resistance to doripenem and resistance to norfloxacin and ciprofloxacin. ATCC BAA-2108 was resistant to all eleven agents, while isolates 66-1 and 00-1 were the most susceptible. Four isolates (46-1, 10-R, 67-1, and 13-1) showed intermediate or resistant responses to oxacillin, penicillin, norfloxacin, and kanamycin. These findings underscore the importance of detecting carbapenem resistance in ***P. aeruginosa*** to guide effective treatment, enhance milk safety, and reduce public health risks from MDR strains.

Keywords: Multidrug resistance, *Pseudomonas aeruginosa*, mastitis, antimicrobial susceptibility test

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INTRODUCTION

Pseudomonas aeruginosa is a pathogenic bacterium that caused mastitis in dairy animals, leading to reduced milk yield, poor quality, and impaired herd development (Abdalhamed et al., 2018). The emergence of multidrug-resistant (MDR) strains has

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increased treatment costs, selective pressure, and risks to both animal and human health (Al-Khazay & Kshash, 2014). Recognized by the World Health Organisation (WHO), *P. aeruginosa* has recently shown resistance to carbapenems. This study aims to investigate carbapenem resistance in *P. aeruginosa* isolated from mastitis-infected milk of Dorper sheeps using the antimicrobial susceptibility disc diffusion test.

MATERIALS AND METHODS

Milk Sampling and Bacterial Isolation

Mastitis-contaminated milk was collected from Agropolitan Besut-Setiu Farm in 5.45° N, 102.88° W in August 2018. The liquid California Mastitis Test (CMT) was used to confirm mastitis (Wan-Azemin et al., 2021, 2022) and 16S rRNA sequencing was performed to identify and validate the *P. aeruginosa* isolates (46-1, 10-R, 67-1, 13-1, 66-1, and 00-1). *P. aeruginosa* strains ATCC 27853 and ATCC BAA-2108 were used as carbapenem control strains.

Antimicrobial Susceptibility

The disc diffusion assay was used to perform the susceptibility antimicrobial test for each isolated strain. 11 antibiotics were assayed on Mueller Hinton Agar (MHA) as listed in Table 1. The CLSI breakpoints determined whether the isolates were sensitive (s), intermediate (i), or resistant (r) to antipseudomonal medicines (CLSI, 2018). Assay was performed in triplicate, and the mean±SD was calculated.

RESULTS AND DISCUSSION

Susceptibility against Conventional Antibiotics

Based on CLSI, (2018) breakpoints, the six *Pseudomonas aeruginosa* isolates showed variable antibiotic susceptibility. Isolate 46-1 was resistant to all agents except doripenem (intermediate). Vancomycin resistance was observed in all isolates, consistent with Swetha et al. (2017). Four isolates (13-1, 46-1, 10-R, and 67-1) also showed intermediate/resistance to penicillin and oxacillin, in line with Swetha et al. (2017). Ciprofloxacin remained effective against most isolates except 46-1 and 67-1, supporting Meng et al. (2020). The variability in resistance may be linked to environmental adaptation and selective exposure (Swetha et al., 2017), while high susceptibility to ciprofloxacin and carbapenems reflects their limited use in dairy mastitis (Aghazadeh et al., 2014).

CONCLUSION

Out of the six isolates, four (46-1, 10-R, 67-1, and 13-1) exhibited a strong MDR phenotype and comparable intermediate/resistance responses to most antibiotics tested. In contrast,

Table 1
Diameter of antibiotics inhibition zone of P. aeruginosa *isolates and ATCC strains (Mean±SD)*

Drugs[a]	Inhibition zone diameter of sample (mm)							
	46-1	**10-R**	**67-1**	**13-1**	**66-1**	**00-1**	**ATCC BAA-2108**	**ATCC 27853**
DOR (10 µg)	17.0±0.0	22.3±0.5	17.0±2.4	31.0±0.8	45.0±0.8	39.0± 0.8	3.3±0.5	44.0± 0.0
MEM (10 µg)	14.3±0.5	20.0±0.0	14.0±1.4	30.3±0.9	43.3±0.5	35.0± 1.6	3.0±0.0	31.3±0.5
FOX (30 µg)	12.3±0.5	22.0±0.0	13.3±0.5	21.3±0.5	34.0±1.4	30.0± 0.5	0.0±0.0	0.0±0.0
OXA (1 µg)	5.0±0.0	17.0±0.0	8.7±0.5	20.0±1.4	34.0±1.4	25.3± 2.5	0.0±0.0	0.0±0.0
PEN (10U)	12.3±0.5	15.3±2.1	18.3±0.9	13.0±1.4	42.3±1.7	37.0± 0.8	0.0±0.0	0.0±0.0
NOR (10 µg)	3.0±0.0	8.0±0.0	10.3±0.9	16.7±0.5	32.0±0.8	24.0± 2.9	5.0±0.8	27.3±2.1
CIP (10 µg)	1.0±0.0	22.3±0.9	14.7±0.5	27.3±2.6	36.3±0.5	27.0±0.5	0.0±0.0	36.3±1.2
KAN (30 µg)	5.3±0.5	6.7±0.5	14.0±0.8	16.0±1.4	29.0±2.9	17.0±1.4	0.0±0.0	0.0±0.0
VAN (5 µg)	5.7±0.5	4.7±0.5	3.3±0.5	12.3±0.5	11.7±0.5	7.3±0.5	0.0±0.0	0.0±0.0
ERY (30 µg)	12.3±0.5	15.7±0.5	17.3±0.5	29.0±1.4	33.3±0.5	23.0±1.6	0.0±0.0	0.0±0.0
LZD (30 µg)	11.7±0.5	20.0±3.6	16.0±1.4	18.0±0.8	37.0±1.6	26.0±2.9	0.0±0.0	0.0±0.0
MHB	0.04±0.0	0.04±0.0	0.04±0.0	0.04±0.0	0.04±0.0	0.04±0.0	0.04±0.0	0.04±0.0

[a]DOR: doripenem; MEM: meropenem; FOX: cefoxitin; OXA: oxacillin; PEN: penicillin G; NOR: norfloxacin; CIP: ciprofloxacin; KAN: kanamycin; VAN: vancomycin; ERY: erythromycin; LZD: linezolid; MHB: Mueller Hinton Broth. Each point is the mean ± SD of three experiments. [b]Color coded; Red: resistant; Yellow: intermediate; Green: susceptible; Grey: not detected

isolates 66-1 and 00-1 were highly susceptible to the same antibiotics. Further research is needed to elucidate the MDR mechanisms of these isolates including genome sequencing which could confirm whether they belong to known *P. aeruginosa* strains or represent novel variants.

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