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Resistance of *Pseudomonas aeruginosa* strains to Rivanol

Abstract. The aim of the study was to determine the sensitivity of two strains of *Pseudomonas aeruginosa* to Rivanol 0.1% (ethacridine). The tests were carried out using the macrodilution method in Brain Heart Infusion (BHI) broth in the concentration range from 1 : 512 to 1 : 1, using an inoculum with a density of 0.5 McFarland. The values of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were assessed. It was found that both strains showed bacterial growth in most of the tested concentrations, and complete inhibition of bacterial growth and bactericidal effect were noted only at the highest concentration used (1 : 1). These results indicate a high resistance of the tested *P. aeruginosa* strains to Rivanol, which may limit its use in the therapy of infections caused by this species. Further research on more effective antibacterial compounds and strategies supporting the therapy of infections caused by multidrug-resistant strains seems to be necessary.

Keywords: *Pseudomonas aeruginosa*, Rivanol, ethacridine, MIC, MBC, resistance

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen of great importance in human and veterinary medicine. It is the cause of numerous hospital infections, especially in patients with reduced immunity, after surgery, during artificial ventilation or with burns [Pang et al. 2019, Qin et al. 2022]. A particular difficulty in treating infections caused by this species is its natural and acquired resistance to a wide range of antibiotics and disinfectants [Namaki et al. 2022, Akiola Sanya et al. 2023]. The resistance of *P. aeruginosa* is due to, among other things, the presence of numerous efflux pumps (MexAB-OprM, MexXY and other systems), low permeability of the outer membrane and the ability to form biofilms, which increase drug tolerance many times over [Egorova et al. 2022]. For this reason, this bacterium has been classified by the WHO as one of the critical priority pathogens, requiring the urgent development of new therapeutic strategies [WHO 2017].

Rivanol (ethacridine) is an acridine dye with antiseptic action, used in the treatment of superficial wounds and ulcers. Its mode of action involves intercalation into the DNA molecule, disruption of its replication and inhibition of nucleic acid synthesis [Jabri et al. 2023]. Despite many years of using in dermatology and surgery, limited effectiveness of Rivanol against Gram-negative bacteria, including *P. aeruginosa* [Namaki et al. 2022], is increasingly observed.

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The aim of this study was to determine the value of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of Rivanol for two strains of *P. aeruginosa* stored in the resources of the Department of Animal Hygiene and Environmental Hazards of the University of Life Sciences in Lublin.

MATERIAL AND METHODS

Bacterial strains

Two clinical isolates of *Pseudomonas aeruginosa* designated as PA-LUB1 and PA-LUB2 used in this study were identical to those previously described in a published study [Targońska-Karasek et al. 2025], which provided their detailed microbiological characterization.

Preparation of inoculum

Bacterial suspensions were prepared in sterile physiological saline (0.85%) and adjusted to a turbidity of 0.5 McFarland standard. A volume of 0.5 ml of this suspension was transferred into a sterile tube containing 4.5 ml of brain heart infusion (BHI) broth and homogenized to ensure uniform distribution.

Determination of MIC and MBC

Rivanol used in the study was a 0.1% solution, with 1 mg of ethacridine lactate in each gram of liquid. MIC and MBC of Rivanol against *P. aeruginosa* were determined by a twofold serial macrodilution technique, adapted from Melville et al. [2003]. Eleven sterile glass test tubes were prepared, each initially containing 5 ml of sterile BHI broth. Into the first tube, 5 ml of undiluted Rivanol solution was added and mixed thoroughly. Subsequently, 5 ml from this tube was transferred into the second tube and mixed, then 5 ml from the second tube was transferred into the third, and so on, through to the eleventh tube. This procedure produced a series of twofold dilutions. An additional tube containing undiluted Rivanol (1 : 0) was also prepared. Each tube was inoculated with 0.5 ml of bacterial suspension. All tubes were incubated at 37°C for 24 hours. MIC was determined as the lowest concentration showing no turbidity. Additionally, all samples were subcultured on Mueller-Hinton agar to determine MBC. All experiments were performed in two independent biological replicates, each in technical duplicate, to ensure reproducibility.

Results were analyzed descriptively due to the qualitative nature of the MIC/MBC data (growth/no growth). Mean values from replicates were used to confirm consistency of results. Data reproducibility between replicates was assessed by comparing the obtained MIC and MBC values for both strains

RESULTS

The results of MIC and MBC determinations are presented in Table 1. Both *P. aeruginosa* strains showed consistent results across all replicates grew over a wide range of Rivanol concentrations from 1 : 512 to 1 : 2. Both the MIC and MBC for the PA-LUB1 and PA-LUB2 strains were only 1 : 1, which indicates a high tolerance of the tested isolates to ethacridine. No differences between replicates were recorded.

Table 1. MIC and MBC of Rivanol – against *P. aeruginosa* strains

Strain	MIC	MBC
PA-LUB1	1 : 1	1 : 1
PA-LUB2	1 : 1	1 : 1

DISCUSSION

The results of this study confirm the high resistance of *P. aeruginosa* to Rivanol, which is consistent with reports of limited effectiveness of acridine dyes against Gram-negative rods [Namaki et al. 2022]. The main mechanisms of this resistance include the presence of MexAB-OprM efflux pumps and reduced permeability of the outer membrane to hydrophobic compounds [Pang et al. 2019, Egorova et al. 2022]. Additionally, the ability of *P. aeruginosa* to form biofilms may significantly limit the penetration of antiseptics and antibiotics into bacterial structures [Egorova et al. 2022].

It should be emphasized that Rivanol in clinical practice is mainly used to treat superficial skin and wound infections. Its use in deep or systemic infections is limited. The obtained MIC and MBC values of 1 : 1 for *P. aeruginosa* strains suggest the need to use practically undiluted concentrations, which is difficult to achieve in conditions that are safe for the patient's tissues. Similar observations have also been shown for other biocides, including octenidine and chlorhexidine, which in the case of multidrug-resistant *P. aeruginosa* strains often require much higher concentrations than those declared by the manufacturers to achieve a bactericidal effect [Namaki et al. 2022].

Modern approaches to combating infections caused by *P. aeruginosa* include the use of efflux pump inhibitors (e.g. phenylalanine-arginine β-naphthylamide) and metal nanoparticles (TiO_2, Ag), which can increase membrane permeability and the effectiveness of existing disinfectants [Qais et al. 2023, Akiola Sanya et al. 2023]. Other strategies include enzymes that break down the biofilm matrix or signaling compounds that inhibit quorum sensing, reducing the expression of virulence factors [Qin et al. 2022].

CONCLUSIONS

The *P. aeruginosa* strains studied were highly resistant to Rivanol, as evidenced by MIC and MBC values at the level of 1 : 1.

These results indicate the limited usefulness of ethacridine in the treatment of infections caused by multidrug-resistant *P. aeruginosa* strains.

Further research is needed on alternative therapeutic strategies, including new antiseptics, efflux pump inhibitors, and methods of disrupting biofilms.

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Ethical statement: No additional ethical approval was required for this study, as the biological sample was collected during routine veterinary medical care while the companion animal was under anaesthesia, with no additional procedures performed beyond standard clinical practice.

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