

Colistin Resistance of *Pseudomonas aeruginosa* Isolated from Snakes

1. Introduction

Antibiotic resistance poses a growing threat to public health ~~all over the globe worldwide~~. Undoubtedly ~~inappropriate, unnecessary~~ prescription is a major ~~issue, and that cause of~~ antibiotic resistance. ~~Antibiotic resistance in agriculture and husbandry animals~~ deserves more focus. ~~Moreover, because the~~ surging level of antimicrobial agents ~~employed by used in~~ veterinary medicine ~~makes have made~~ animals ~~today~~ potential reservoirs of resistant bacteria [1].

~~Categorized as~~ Infection caused by *Pseudomonas aeruginosa*, an opportunistic pathogen, ~~nonetheless, Pseudomonas aeruginosa infection~~ could be lethal in ~~the an~~ immunocompromised population [2]. ~~Yet~~ ~~However,~~ the ~~pivotal~~ ~~major~~ problem lies in the intrinsic *P. aeruginosa* antibiotic resistance ~~of P. aeruginosa that, which~~ counteracts the effects of medical treatments [3]. ~~To~~ ~~current knowledge,~~ *P. aeruginosa* is more likely to be an environmental species ~~and passed on that is transmitted~~ by animal reservoirs, ~~thesuch as~~ snakes, ~~for instance~~ [3]. ~~In fact, the latest report from~~ The Centers of Disease Control ~~illustrates that incidence of snakebites reaches~~ ~~has estimated~~ more than 1,000 ~~eases~~ ~~incidences of~~ snakebites annually [4], ~~and implicates underestimated suggesting that the role of~~ snakes in *P. aeruginosa* ~~of snake origin infection is underestimated~~.

However, ~~little is detailed about the determination information~~ regarding the susceptibility of antibiotics that are widely administered within ~~the~~ human population, such as piperacillin/tazobactam and meropenem, ~~is limited~~ [5]. ~~Since~~ ~~Because~~ disease-free snakes might be carriers ~~as well,~~ captive ~~ones~~ snakes of commercial origin and ~~those~~ from research ~~institution~~ ~~institutions~~ were also ~~enrolled~~ ~~included~~ in our study. In ~~brief,~~ ~~this study, we performed~~ sensitivity testing of *P. aeruginosa* from snakes' oral cavities and subsequent molecular typing of resistance genes ~~will be outlined in the following paragraph~~, with further implication of clinical translation and public health responses.

2. Materials and Methods

2.1. *Pseudomonas aeruginosa* Strains

The study ~~was implemented based on~~ ~~included~~ 58 *P. aeruginosa* isolates from disease-free captivated snakes, wild snakes at Endemic Species Research Institute (ESRI), and clinical cases at NCH University Veterinary Medicine Teaching Hospital. All samples were ~~collected~~ ~~obtained~~ through sterilized swabbing ~~of oral cavities of the snakes~~ and ~~stored in the transport media on~~ ~~transported to~~ the ~~way to~~ microbiology laboratory ~~in transport media~~. Following ~~the~~ aerobic culture routine, the isolates were plated ~~on~~ ~~on~~ cetrimide agar and incubated at 35°C in 5% CO₂ and 95% air for 48 hours. ~~Based on the~~ ~~The~~ acquired colonies, *Pseudomonas P. aeruginosa* ~~was~~ ~~colonies~~ ~~were~~ further ~~determined by~~ ~~analyzed on the basis of~~ colony morphology, Gram

Comment [Editor1]: Please review the change here, and ensure that your intended meaning has been preserved.

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~~stainstaining~~, oxidase test, and API ID 32 GN strips (bioMérieux, Marcy l'Etoile, France).

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2.2. Antimicrobial Susceptibility Testing

~~The minimum~~Minimum inhibitory concentrations (MICs) were identified by Clinical Laboratory Standards Institute (CLSI) [6]. Targeting *P. aeruginosa*, cation-adjusted Müller–Hinton broth was ~~engaged to~~used in subsequent serial twofold dilutions of representative antimicrobial agents. ~~*P. aeruginosa*~~ suspension was then adjusted to ~~the~~a turbidity of 0.5 McFarland standard of ~~*P. aeruginosa*~~ ATCC27853, and 100 μ L of the bacteria with an inoculum density ~~at around of~~approximately 10^5 CFU/mL was further transferred onto 96-well plates. After incubation at 37°C for 24 hours, the MICs of the respective antibiotics were ~~then~~ categorized into susceptible, intermediate, and resistant ~~based on the interpretation table within according to~~ CLSI ~~guidance~~guidelines. The following antimicrobials were ~~employed~~used in the study: penicillins (piperacillin/tazobactam), cepheims (cefotaxime), aminoglycosides (gentamicin and amikacin), lipopeptides (colistin), monobactams (aztreonam), and carbapenems (meropenem).