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Comparative evaluation of prevalence of extended spectrum beta lactamase genes in *Enterobacteriaceae* isolates from wild-life

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Abstract
Antimicrobial Resistance (AMR) remains a persistent concern in the One Health framework. Inappropriate antibiotic use has given rise to drug-resistant pathogens, challenging the treatment of infections. This resistance occurs when microorganisms withstand the effects of drugs. Contributing factors include overuse, improper dosage, and inappropriate agricultural practices. Strategies to address this issue encompass promoting responsible drug use, surveillance, education, research, and fostering international collaboration. A total of 285 isolates were screened, comprising 53 *E. coli* and *Shigella*, 230 *Salmonella enterica*, 1 Yersinia, and 1 Enterobacter (Figure 1). Beta-lactamase genes, including blaEC, blaTEM-1, blaCARB, blaA, and blaATC-27, were identified among the isolates. BlaEC was most common in E. coli and Shigella, while blaCARB-2 and blaTEM-1 predominated in Salmonella enterica serovars, specifically (I 4,[5],12:i:-) and Typhimurium. BlaA was found in Yersinia enterocolitica, and blaACT-27 occurred in Enterobacter. Beta-lactamase genes were more frequently associated with the ESKAPE group of pathogens, such as *E. coli* and Enterobacter, than *Salmonella enterica* serovars. A total of 16 *Salmonella enterica* serovars were recorded, including Enteritidis, Cholerasuis, Typhimurium, Derby, Ball, Coeln, Agona, Infantis, Goldcoast, London, Indiana, Anatum, Pensacola, Sangalkam, I 14,[5],12:i:-, Kottbus, and Newport. The prevalence of beta lactamase genes and their frequency in wildlife highlights the risk of its adverse effects like treatment failure, global and local spread in wildlife. This also pose a threat to the endangered species. The absence of metalo beta lactamases is indicative of less use of carbapenems in wildlife.

Keywords: *Enterobacteriaceae*, antimicrobial resistance, *E. coli*

Introduction
Antimicrobial Resistance (AMR) is an ongoing concern within the One Health framework. Inappropriate antibiotic use has led to the emergence of drug-resistant pathogens. This resistance occurs when microorganisms defy the effects of drugs, creating challenges in treating infections. Overuse, improper dosage, and inappropriate agricultural practices contribute to AMR. To combat this issue, strategies involve promoting responsible drug use, surveillance, education, research, and international collaboration. These efforts are crucial for preserving treatment effectiveness and managing infectious diseases. AMR is not confined to human populations; it extends to wildlife. The use of antimicrobial agents in veterinary medicine, agriculture, and aquaculture contributes to resistance in various wildlife species. Wildlife can be exposed through contaminated water, soil, or food sources, stemming from human activities like antibiotic use in agriculture or pharmaceutical release into the environment. Veterinary use of antimicrobial agents, runoff from agriculture, and improper pharmaceutical disposal can introduce these agents into wildlife habitats, impacting microbial communities. This resistance in wildlife can disrupt ecosystems and potentially transfer resistant genes to human or other animal populations, contributing to the global spread of AMR. Moreover, the growing frequency of international travel and trade has played a role in the swift global dissemination of antimicrobial resistance (Laxminarayan *et al*., 2013) [1].

Nevertheless, the contribution of wildlife to the emergence of antibacterial resistance may be underestimated. The first instance of antibacterial resistance in wildlife was observed in Japanese wild birds, where chloramphenicol resistance was identified in *E. coli* isolates. Since then, the prevalence of resistant bacteria in wild animals has been consistently reported across diverse species and various geographic locations. Furthermore, notable antimicrobial resistant pathogens such as MRSA, vancomycin-resistant *Enterococci*, *Salmonella* spp., *Vibrio cholerae*, and *Campylobacter* spp. have been documented in wildlife. These findings emphasize the significance and complexity of the role played by wildlife, typically not directly exposed to antibiotics, in the transmission of resistant bacteria (Sato *et al*., 1978; Loncaric *et al*., 2013; Porrero *et al*., 2014; Drobni *et al*., 2009; Sellin *et al*., 2000; Lee *et al*., 2011; Aberkane *et al*., 2015; Weis *et al*., 2016) [2-9]. The discovery of successful clones in humans, domestic animals, and wildlife suggests the potential for interspecies transmission of ESBL-producing isolates. Nevertheless, the horizontal transfer facilitated by mobile elements, including insertion sequences and plasmids, stands out as a primary mechanism for the global dissemination of ESBL (Wang *et al*., 2017) [10].

Addressing this issue requires a comprehensive One Health approach, acknowledging the interconnectedness of human, animal, and environmental health. Antimicrobial resistance (AMR) poses severe threats to health, agriculture, and the environment. It results in ineffective infection treatments, heightened morbidity and mortality, economic strain on healthcare systems, and diminished efficacy of medical interventions. AMR contributes to the spread of resistant strains, impacting agriculture and global health security. The challenges include limited treatment options, a dearth of new antimicrobials, and the interconnection of human, animal, and environmental health. Effectively addressing AMR requires global cooperation, prudent antimicrobial use, research for new treatments, and public awareness to prevent serious repercussions for global health.

Materials and Methods

Data collection and processing

The isolates were screened from NCBI National Database of Antibiotic Resistant Organisms (NDARO) https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/. The "Browse Genomes with AMR genotypes or Phenotypes" search option was opted to screen isolates from wildlife origin. A total of 285 *Enterobacteriaceae* isolates from wildlife origin were screened with filters like Isolation source (wildlife), Organism group, AMR genotypes and AST phenotypes for the presence of beta lactam genes *viz. BlaTEM*, *blaCTXM*, *blaCMY*, *blaNDM*, *blaKPC*, *blaIMP*, *blaCARB*, *blaOXA*, *blaSHV*, *blaEC* etc. Under the Organism group, Keyword "wildlife" gave results such as "wildlife", "wildlife faeces", "faeces from giant Panda from shaanxi rare wildlife", "grey seal pup", "wildlife faecal samples in bovine feedlots", "wildlife from national animal disease center"and all the options were selected Thereafter the prevalence of each organism group in wildlife was recorded. Under organism group, *E. coli* & *Shigella*, *Salmonella enterica*, *Enterococcus faecalis*, *Enterobacter hormaechi*, *Campylobacter jejuni*, *Pasteurella multocida* and *Yersenia enterocolitica* came as isolates obtained from wildlife sleected. But only the members of *Enterobacteriaceae viz. E. coli* and *Shigella*, *Salmonella enterica*, *Enterococcus faecalis*, *Enterobacter hormaechi* and *Yersenia enterocolitica* were selected. The presence of beta lactamase genes of each category *viz*. Type A, B, C and D were investigated among the *Enterobacteriaceae* isolates. The interpretations are recorded in the graphical format for each group.

Results

A total of 285 isolates comprising of 53 *E. coli* and *Shigella*, 230 *Salmonella enterica*, 1 *Yersinia* and 1 *Enterobacter* isolates were screened (Figure 1). The beta lactamase genes *viz. blaEC*, *blaTEM-1*, *blaCARB*, *blaA*, *blaATC-27* were recorded among the isolates. *BlaEC* was the most common betalactamase genes found in *E. coli* and *Shigella* group whereas *blaCARB-2* and *blaTEM-1* most commonly occurred in *Salmonella enterica* serovars *viz*. (I 4,[5],12:i:-) and Typhimurium, *blaA* in Yersinia enterocolitica and *blaACT-27* in *Enterobacter*. Beta lactamase genes were more frequently associated with ESKAPE group of pathogens *viz. E. coli*, *Enterobacter* rather than *Salmonella enterica* serovars. A total of 16 Salmonella enterica serovars were recorded *viz*. Enteritidis, Cholerasuis, Typhimurium, Derby, Ball, Coeln, Agona, Infantis, Goldcoast,1 London, 1 Indiana,1 Anatum, 1 Pensacola Sangalkam, I 14,[5],12:i:-, Kottbus and Newport. The data recorded is represented in the form of a table (Table no 1).

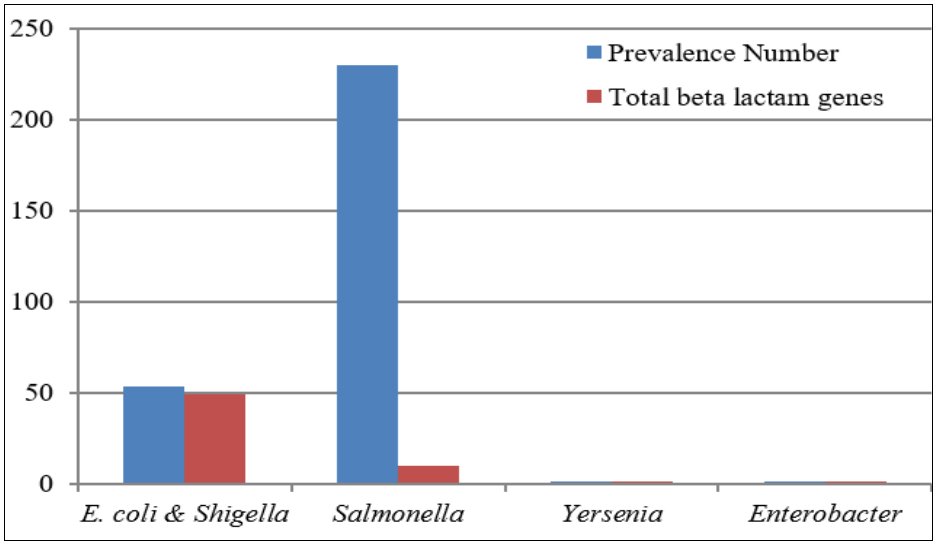

Fig 1: Prevalence of *Enterobacteriaceae* in wildlife samples

Table 1: Prevalence of Beta lactamase genes among various isolates of *Enterobacteriaceae* family

S. No.	Organism Group	Serovar	Total	Source	Total	Beta lactamase gene	Total	Prevalence in Organism Group (%)
1	***E. coli* and *Shigella***			**Wildlife**	**53**	**blaEC**	**49**	**92.45**
2	*Salmonella enterica*	Enteritidis	60	Wildlife	230	blaTEM blaCARB-2	10	4.43
		Cholerasuis	89					
		Typhimurium	53					
		Ball	7					
		Coeln	1					
		Agona	1					
		Infantis	2					
		London	1					
		Indiana	1					
		Anatum	1					
		Pensacola Sangalkam	1					
		Goldcoast	1					
		Derby	2					
		Newport	1					
		Kottbus	1					
		I 14,[5],12:i:-,	8					
3	*Yersinia enterocolitica*			Wildlife	1	blaA	1	100
4	*Enterobacter*			Wildlife	1	blaACT-27	1	100

Discussion

BlaEC type beta lactamase belongs to AmpC type beta lactamase category or class C beta lactamase. These enzymes, known as cephalosporinases, hydrolyze a wide range of antibiotics, including commonly used penicillins, cephalosporins, oxyimino-cephalosporins, and monobactams. Bacterial resistance to these antibiotics is a significant clinical concern, as continuous enzyme production can raise minimum inhibitory concentration (MIC) values beyond clinical breakpoints. The transition from inducible to continuous enzyme production during antibiotic treatment poses a risk of treatment failure, particularly in cases of sepsis, potentially leading to patient death. Traditional beta-lactamase inhibitors like clavulanic acid and sulbactam have no effect on this enzyme group (Jiri *et al*., 2023) [11]. The blaCARB-2 gene, previously known as blaPSE-1, is commonly found within the chromosomal cassette. Notably, this carbenicillinase gene has the ability to move across different lineages of S. enterica serovars globally, contributing to the spread of antimicrobial resistance (AMR) (Lopes *et al*., 2016; Huovinen *et al*., 1991, Kamolvit *et al*., 2015) [12, 13, 14]. Highly pathogenic Yersinia enterocolitica biovar 1B produces two distinct β-lactamases, BlaA and BlaB. Mutants from a representative biovar 1B isolate were examined to gauge the impact of BlaA and BlaB on susceptibility to broad-spectrum β-lactam antibiotics. Results show that BlaA significantly influences susceptibility to penicillins and cephalosporins, while the contribution of BlaB is less profound, primarily affecting cephalosporin susceptibility (Bent and Young, 2010) [15]. Among the acquired AmpC genes, bla_{ACT} types are commonly identified in *E. cloacae* complex. These are plasmid mediated AmpC betalactamases effectively hydrolysing cephalosporins. However, any mutations in the narrow spectrum beta lactamases can widen its spectrum making it resistant to many other antibiotics (Tickler *et al*., 2023) [16]. BlaTEM belong to group B beta lactamases that are serine proteases. The absence of metalo beta lactamases in the isolates of wildlife origin indicates their less frequent use in wild life. However, the reports on the presence of metalobeta lactamases in environmental samples does not rule out the possibility of acquisition of antimicrobial resistance from environment.

Conclusion

Antimicrobial resistance (AMR) in wildlife remains an underexplored and less investigated area. However, it is crucial to further research in this field as it not only reveals the existing burden of AMR but also contributes to the global dissemination of AMR genes, both within and outside the country through exports. Addressing AMR is a complex challenge that requires an enhanced understanding of its spread and the reduction of unnecessary and widespread use of antimicrobials in everyday life.

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