

The need for efforts to obtain high quality evidence in a one health approach.

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Abstract

The progressive increase of antibiotic resistance poses an alarming threat on public health worldwide. Although antibiotic resistance is a problem of human health, a one health approach to tackling antibiotic resistance is required, due to the effect of animal and environment on human health. To exactly estimate the impact of animal and environment on the spread of antibiotic resistance in human, scientific evidence with high quality is required. Molecular analysis of antibiotic-resistant bacteria through high-resolution, such as whole-genome sequencing, is suitable for obtaining credible evidence in examining the transmission of strains or genetic mobile elements responsible for antibiotic resistance among human, animal, and environment. In this paper, we discuss many observational studies using the whole-genome sequencing on various isolates collected from human, animal, and environment. These analyses suggest that more extensive surveillance based on whole-genome sequencing is required to accurately assess the potential threat of animal and environment on the spread of antibiotic resistance in human and that the effort to overcome various limitations in obtaining higher quality evidence is required.

Keywords: Antibiotic resistance, One health approach, Whole-genome sequencing, Animal, Environment.

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Introduction

From the first introduction in the market of a commercial antibiotic, antibiotics have been used in veterinary medicine as well as human medicine [1]. In addition to therapeutic purposes, antibiotics administered to food animals have been also used as growth-promoting agents to obtain sufficient amount of food [2]. Antibiotics for non-therapeutic purpose are generally supplied to food animals through feeding at very low concentrations for a long time [3,4]. Such antibiotic usage is known to be a dangerous practice, because antibiotics at low concentrations are able to generate selective pressure which could enrich antibiotic-resistant bacterial populations [5,6]. Therefore, antimicrobial growth promoters were banned in many European countries and the United States [4,7], but clinically important antibiotics are still being fed to food animals for growth promotion in some countries [3,4]. Several antibiotics have been used only in veterinary medicine, but some of them are structurally very similar to clinically important antimicrobial agents, such as fluoroquinolones and macrolides [8]. Most of commercial antibiotics have been used in both human and veterinary diseases for a long time [4].

These indiscriminate uses of antibiotics in livestock settings have caused the emergence of antimicrobial resistance.

Antimicrobial resistance has been considered a one health issue [9]. One health is an emerging concept that the human health is connected to the health of animal and environment. There is general scientific consensus that the use of antibiotics in veterinary medicine exerts selective pressure for the emergence of antimicrobial resistance in human [10]. Antimicrobial-resistant bacteria selected by antibiotic use in veterinary medicine could spread to humans through foods, direct contact with animals, or environmental pathways [10,11]. However, to know why the recent antibiotic resistance crisis requires One Health approach, scientific evidence with high quality is required. Molecular analysis of antibiotic-resistant bacteria through high-resolution, such as whole-genome sequencing, has scientific merits in the elucidation of the connection between human health and animal (or environmental) health [12-14].

A recent study performed by Alicia et al. reported a retrospective, whole-genome sequencing study analyzing 288 *Salmonella enterica* serotype *Typhimurium* isolates obtained between 1911 and 1969 from 31 countries and from various

sources, including human, animal, and food [15]. They showed the existence of ampicillin-resistant *S. enterica* serotype *Typhimurium* strains in human beings before ampicillin was released to the market. The authors suggested that the non-clinical use of narrow-spectrum penicillins, such as penicillin G, may exert selective pressure on the emergence of ampicillin-resistance *S. enterica* serotype *Typhimurium* in human. Based on this assumption, they highlighted the need for a One Health approach. To understand the connection between human health and animal (or environmental) health, we analysed all reported whole-genome sequencing studies examining the effect of animal and environment on antibiotic resistance of human. The analysis results suggest the following important aspects: (i) requirement for more extensive and observational evidences for the transmission of various strains or genetic mobile elements responsible for antibiotic resistance among human, animal, and environment; (ii) requirement for Randomized Controlled Trials (RCTs).

Observational Evidences for the Transmission of Antibiotic Resistance among Human, Animal, and Environment

There are many observational studies using the whole-genome sequencing tool to analyse the possibility of the transmission of genetic mobile elements or strains responsible for antibiotic resistance among human, animal, and environment (Table 1). The whole genome sequencing studies showed that the same antibiotic-resistant foodborne and zoonotic pathogens, such as *S. enterica*, *Staphylococcus aureus*, and *Campylobacter* sp., are detected in both human and animals [16-28]. For example, cefoxitin-resistant *S. enterica* serovar Heidelberg strains with 0 to 4 Single Nucleotide Variations (SNVs) (i.e. almost genetically identical strains) are identified in human, abattoir poultry, and retail poultry isolates [18]. This result suggests that the transmission of cefoxitin-resistant *S. enterica* serovar Heidelberg strains among human, abattoir poultry, and retail poultry sources may happen. The clonal transmission of opportunistic pathogens, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Clostridium difficile*, was also detected [29-38]. The whole genome sequencing of *C. difficile* strains, which are a commensal organism and a pathogen in both human and animal [39], showed that *C. difficile* isolates with identical (no SNP differences) and almost identical (less than two SNP differences) are detected in both farmers and pigs in the Netherlands [34]. Many β -lactam-resistant *E. coli* strains identified in human samples were also detected in animals [29,31,35-38] and environment [35,40,41]. These results indicate the complex transmission of β -lactam-resistant *E. coli* strains among human, animal, and environment.

In addition to the clonal transmission, the transmission of mobile genetic elements responsible for resistance of various antibiotics, such as β -lactam, colistin, and tetracycline, was also detected (Table 1). Colistin has been used in veterinary medicine for the prevention of infections and growth promotion for a long time, but it recently remains one of the last-resort drugs to treat infections caused by multidrug-

resistant gram-negative pathogens, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *E. coli*, and *K. pneumoniae* [42]. Since a first report for the presence of the *mcr-1* gene responsible for plasmid-mediated colistin resistance in commensal *E. coli* isolated in pig farms in China [43], the *mcr* genes have been reported among various bacterial pathogens worldwide [42]. Many reports showed that the same plasmids containing the *mcr* genes are detected in *E. coli* isolates from human and animals [44-51]. For example, some colistin-resistant *E. coli* strains isolated from commercial chicken meat in Brazil carried an IncX4 plasmid containing the *mcr-1* gene [47] and the sequence of this plasmid showed 100% identity to that of another IncX4 plasmid (pICBEC72Hmcr), which is detected in a colistin-resistant *E. coli* strain isolated from human in Brazil [52]. Notably, two IncX4 plasmids originating from environmental samples in Germany were almost identical to the pICBEC72Hmcr plasmid [53]. These results suggest that the *mcr-1*-containing IncX4 plasmids may be globally distributed in human, animal, and environment.

Scope for Further Studies

First, observational studies using whole-genome sequencing showed that the clonal transmission between human and animal is investigated in several antibiotic-resistant bacteria, including foodborne or zoonotic pathogens and commensal bacteria. However, these investigations are focused on several bacteria, such as *S. aureus*, *E. coli*, and *S. enterica*. It is required to examine whether the clonal transmission of other clinically important bacteria between human and animal is possible or not. To accurately assess public health impacts of antibiotic resistance in animal and environment, the continued monitoring based on whole-genome sequencing of bacterial isolates in human, animal, food and environmental sources is required. The study of Alicia et al. showed the existence of ampicillin-resistant *S. enterica* serotype *Typhimurium* strains in human beings before ampicillin were released to the market. The authors suggest that the non-clinical use of narrow-spectrum penicillins, such as penicillin G, may exert selective pressure on the emergence of ampicillin-resistance *S. enterica* serotype *Typhimurium* in human. This result underlines the importance of One Health approaches and the merit of whole-genome sequencing. Therefore, the retrospective, whole-genome sequencing analysis of a large number of isolates collected from human, animal, or environment have to be continued.

Second, because observational studies provide low quality evidence, RCTs are needed for high quality evidence [54]. Many RCTs evaluating the effect of antimicrobial agents and probiotics on human health have provided high quality evidence on their effectiveness and safety [55-57]. However, there is no RCT to evaluate the transmission of antibiotic-resistant strains or genetic mobile elements between animal and human [58]. RCTs evaluating the transmission of antibiotic resistance from animal or environment to human are generally not feasible [59]. Nevertheless, the effort to overcome various

limitations in obtaining high quality evidence is required, due to the overwhelming and urgent threat of antibiotic resistance on public health. In conclusion, these efforts will provide an

important scientific basis for exactly estimating the impact of animal and environment on the spread of antibiotic resistance in human.

Table 1. Observational studies analyzing the possibility for the transmission of strains or genetic mobile elements responsible for antibiotic resistance among human, animal, and environment through whole genome sequencing.

Source	The transmission of strains or genetic mobile elements	Antimicrobial agent	Species	References
Animal and human	Identification of the same antimicrobial-resistant strain	β-Lactam	<i>Staphylococcus aureus</i>	[17,19-22,24-26]
			<i>Salmonella enterica</i>	[18,23,27]
			<i>Escherichia coli</i>	[29,31,35-38]
			<i>Klebsiella pneumoniae</i>	[30]
			<i>Campylobacter sp.</i>	[28]
			<i>Enterobacteriaceae</i>	[59]
		Quinolone and fluoroquinolone	<i>Salmonella enterica</i>	[16]
			<i>Escherichia coli</i>	[32]
		Sulfamethoxazole	<i>Salmonella enterica</i>	[16]
		Tetracycline	<i>Clostridium difficile</i>	[34]
		Multidrug	<i>Escherichia coli</i>	[33]
		Identification of the same genetic mobile element carrying the antibiotic resistance gene	β-Lactam	<i>Escherichia coli</i>
<i>Salmonella enterica</i>	[18,27,63]			
Colistin	<i>Escherichia coli</i>		[44-51]	
	<i>Salmonella enterica</i>		[64]	
Tetracycline	<i>Staphylococcus aureus</i>		[65]	
Environment and human	Identification of the same antimicrobial-resistant strain		β-Lactam	<i>Escherichia coli</i>
		<i>Enterobacteriaceae</i>		[59]
		Multidrug	<i>Escherichia coli</i>	[40]
	Identification of the same genetic mobile element carrying the antibiotic resistance gene	β-Lactam	<i>Enterobacteriaceae</i>	[67]
		Colistin	<i>Escherichia coli</i>	[53]

Conflict of Interests

The authors declare that they have no competing interests.

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