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ANTIBACTERIAL RESISTANCE: A COMPARATIVE STUDY BETWEEN CLINICAL AND ENVIRONMENTAL ISOLATES

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ABSTRACT

This comparative study was carried out to investigate the presence of antibiotic sensitive and resistant bacteria isolated from different environmental samples of Bangladesh with specific clinical strain obtained from ICDDR, B .The environmental isolates were collected from the month April 2015 to September 2015 from different locations of Dhaka city to perceive the propensity of these environmental isolates to develop antibiotic resistance at different time periods of a year. The bacterial isolates were identified on the basis of the standard cultural, morphological and biochemical characteristics. Antibiogram was done to identify the antibiotic susceptibility of the isolates according to CLSI guidelines. The result of this study showed that all the environmental isolates have become resistant to the tested antibiotics, and some of them have become multi-resistant to these therapeutic agents whereas all the clinical isolates showed the opposite result. The study helps to predict the future emergence and to guide the development of strategies towards counteracting this resistance. Therefore periodic and comprehensive survey of antibiotic resistance in the environmental bacteria is required.

Keywords: Antibiotic resistance, antibiogram, environmental isolates, emerging pathogen.

Introduction

It has long been established that, natural selection shapes the evolution of DNA based organisms, through the course of survival or extinction within the dynamic environment of the planet. Through breakthrough scientific discoveries, the all-connecting symbiotic nature of all living things have become more and more evident. With this knowledge it is now understood far better than ever that as humans, with a huge and sustained footprint on the environment, we have become a substantial influence in the context of evolution. This idea is a bit hard to grasp as most of the noticeable evolution takes place over thousands to millions of years, but when it comes to microorganisms, it is fairly observable within human lifespan that these single celled creatures are adapting to the direct or indirect interactions with us and the environment as a whole, with ferocious rapidity.

A growing interest amidst the community of microbiologists is to study this dynamic relationship as it is imperative to maintain our bloated life expectancy bestowed by the era of antibiotics. In this trend, common disease-causing microorganisms and our methods of eradicating or controlling them are subjects of repeated rigorous scientific experiments. These studies are carried out by various pharmaceutical companies, organizations for disease control, genetic engineers, biotechnologists, evolutionary micro-biologists and every other group that have a vested interest in improving and maintaining the quality of human life.

A humongous number of organisms are associated with a variety of infectious diseases, and are considered to be human pathogens. Along with the pre existing ones some pathogens that have only been recognized very recently are menacing human health. Such pathogens are largely accountable for a massive number of deaths every year. As many new diseases are held responsible for this global burden, which is taking its toll on human health, it is also true for diseases that had been declining over the past few years [1]. The re emergence of these diseases is associated with certain transmission routes as well different and a wide range of hosts.

Antibiotics, since its invention have been vital in the fight against infectious diseases that are caused by various bacteria as well as other microbes. A matter of great misery is that although we have all these advanced antibiotics at our disposal now, reports have shown that no antibiotic can actually last effective for too long. As a result, resistance of the pathogens to common therapeutic agents has been increasing in recent years. Thus, a mere cultural contaminant or a harmless microbe can eventually become serious pathogen over time [2].

The abundance and indiscriminate use of the commercial antimicrobial drugs that are commonly used in the treatment of infectious diseases is the leading reason behind multiple drug resistance in human pathogenic microorganisms that has been developed over the past years [5]. This development of antibiotic resistance has a number of favoring factors, that includes the characteristics of the hosts, the usage of antibacterial agents, the specific nature of the relationship between bacteria and antibiotics, and some environmental factors [3]. Since the bacterial pathogens have the ability to rapidly evolve, this helps them to come up with new ways to dodge the host defenses and become resistant to the antibiotic treatments. It is a matter of great concern that more and more pathogens are showing resistance to multiple drugs. In addition the high levels of antibiotics used in humans and animals now a days have amplified the emergence of these antibiotic resistant strains that are the main concern of this study [4].

Although the pharmaceutical industries have produced a number of new antibiotics in the last few decades, simultaneously the resistance of the microorganisms to these drugs has also increased. Due to such increasing resistance in microbes and synthetic antibiotic side effects, it is now necessary to access resistance pattern of pathogenic organisms against standard antibiotics to develop therapeutic alternatives.

On the basis of the above context, the objectives of the present study are:

- to evaluate the degree of antibacterial activity considering the diameter (mm) obtained for the zone of inhibition on each of the replicate agar plate.
- to record the resistance pattern of both clinical and environmental samples for a comparative analysis.

Research procedure:

Bacterial culture maintenance

In this study, five standard clinical strain of *Bacillus cereus, E. coli K12, Salmonella typhi, Staphylococcus aureus,* and *Shigella flexneri* were used. All these species were obtained from ICDDR, B (International Center for Diarrheal Disease Research, Bangladesh).

Reference bacterial strains were identified routinely to distinguish each organism by subculturing on recommended selective media. The cultural properties of each organism were inspected and recorded.

Maintenance environmental sample

Different types of environmental samples were collected from different sources during the month of October, 2014 (Table 1). Throughout October, the daytime temperatures generally reached as high as up to around 30°C, which is 86°F. At night, the average minimum temperature dropped down to around 21°C, which is about 70°F. The average daily relative humidity for October was around 84%.

Table 1: Source of environmental samples

Environmental Sample	Collected from
Chicken feces	a chicken farm located at Banani
Cow dung	a farm located at Gulshan
Handmade salad	a restaurant located at Mohakhali
Human sewage	The river Buriganga

Biochemical Identification

Biochemical tests were performed with all specific standard and environmental isolates developed in specific media according to the standard methods described in Microbiology Laboratory Manual [6]. Before starting the process of any biochemical identification test, all the bacterial cultures were grown on nutrient agar plates in the incubator at 37°C.

Antibiotic susceptibility test of the standard clinical and environmental strains of bacteria

In antibiogram (disc diffusion) assays, the response of each employed growing population of microorganism to the standard antibiotic was investigated to record the potential antibacterial activity.

In this study, the effectiveness of thirteen different antibiotics was determined. They are listed below in the Table 2:

Table 2: Provided Antibiotic Discs

Antibiotic	Disc Identification Number
Sulfamethoxazole / Trimethoprim	(SXT 25)
Cefoxitin	(FOX 30)
Pefloxacin	(PEF 5)
Ciprofloxacin	(CIP 5)
Erythromycin	(E 15)
Gentamicin	(CN 10)
Kanamycin	(K 30)
Streptomycin	(S 10)
Cefuroxime Sodium	(CXM 30)
Nalidixic Acid	(NA 30)
Oxacillin	(OX 1)
Chloramphenicol	(C 30)
Nitrofurantoin	(F 300)

The antibiotis discs were placed on surface of MHA plates cultured with clinical and environmental isolates and incubated overnight at 37°C for 24 hours. Then diameter of inhibition zones was measured in mm.

McFarland standards were used as reference to adjust the turbidity of any given bacterial suspension. This was done to make sure that the number of bacteria was within a given range so as to standardize the microbial testing. This would also help avoid any error in result, because if the suspension was too heavy or too diluted, an erroneous result might occur for any given anti microbial agent, which in this study, was the antibiotic discs.

Results

All the clinical and environmental strains were confirmed by their cultural properties upon streaking on the respective selective media and appropriate biochemical tests.

Selective antimicrobial activity test by means of antibiogram method

The standard disc diffusion test was done with all the provided antibiotics (Table 2) against five clinical and isolates of five selected environmental strains to identify their resistance pattern. The interpretive categories were defined according to the zone diameter of inhibition.

All the clinical strains showed significant susceptibility to all the antibiotics except for OX1 (Figure 9). *Salmonella typhi* showed the maximum level of susceptibility to C30, which was around 34mm in diameter, whereas, both *E. coli* K12 and *Bacillus cereus* showed the highest level of susceptibility to CIP5 which was measured to be 40mm and 28mm in diameter respectively. *Bacillus cereus* also showed the similar level of susceptibility (28mm in diameter) to E15 .In case of *Shigella flexneri*, the utmost level of susceptibility was observed when CXM30 and FOX30 were applied to the disc diffusion test (26mm in diameter). In case of *Staphylococcus aureus*, the maximal level of susceptibility was showed by PEF5, which was around 32mm in diameter. Antibiotic susceptibility test results for clinical strains are represented in Table 3 (Annex 1) and Figures 1 below.

A significant level of resistance was observed when all the standard antibiotics were applied against several isolates of the selected environmental strains: *Bacillus cereus* collected from salad and human sewage samples; *Shigella flexneri* collected from chicken feces; and *Salmonella typhi* collected from human sewage sample. The *E. coli* collected from salad and bovine samples (cow dung), and *Staphylococcus aureus* collected from human sewage and chicken feces samples, all showed resistance to at least one or more than one antibiotics.

In case of *B. cereus* collected from salad sample, the isolate S3 showed resistance to the antibiotic FOX30, and the isolate S6 was resistant to PEF5 and F300, whereas, the clinical strain of *B. cereus* showed significant zone of inhibition to these same antibiotics, which were measured to be 24mm, 25mm and 22mm in diameter respectively . The isolate H1 of *B. cereus* collected from human

sewage sample showed resistance to the antibiotic PEF5 and H2 showed resistance to CIP5 and S10. On the other hand their clinical strain showed zone of inhibition to these same antibiotics and were 25mm, 28mm, and 20mm in diameter respectively Figures 2 .

The isolate C3 of *S. flexneri* collected from chicken feces showed resistance to two different antibiotics, FOX30 and E15; whereas the susceptibility of their clinical strain to these antibiotics was 26mm and 20mm in diameter respectively. In case of *S. typhi*, which was collected from human sewage sample, the isolate H1 showed resistance to the antibiotics FOX30, E15, and S10, and the isolate H3 showed resistance to the antibiotics SXT25, PEF5, E15, and C30. The clinical strain of *S. typhi* was vulnerable to all these antibiotics.

The clinical strain of *E. coli* was significantly vulnerable to all the antibiotics, yet three different isolates of *E. coli* collected from salad samples showed resistance to three different antibiotics each (Figure 8) and four isolates collected from the bovine sample (cow dung) showed resistance to three and four different antibiotics each (Figure 7) .

In case of environmental strain of *Staphylococcus aureus*, the result was astounding as the clinical strain of *Staphylococcus aureus* was susceptible to all the test antibiotics except one, having large zones of inhibition measured to be as high as 28-32mm in diameter .Where as different isolates of *S. aureus* collected from human sewage sample and chicken feces showed resistance to as many as four and eight different antibiotics.

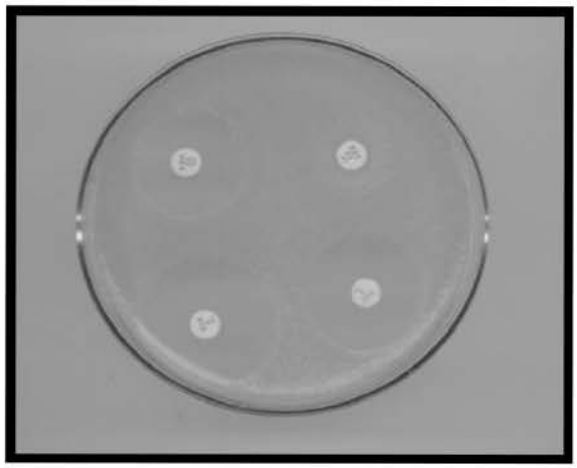

Fig. 1: Effect of provided antibiotics on clinical strain of B. cereus

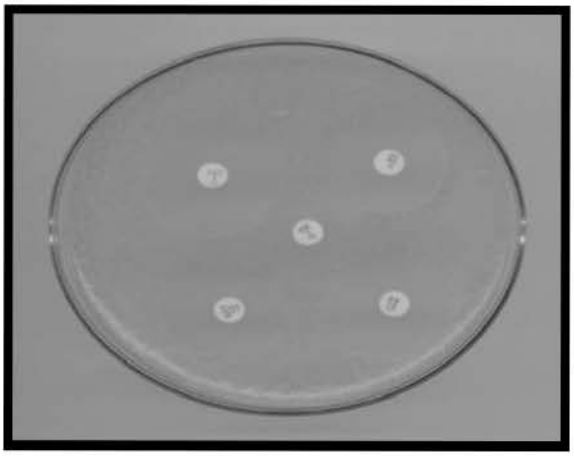

Fig. 2: Effect of provided antibiotics on B. cereus S3 isolate collected from salad sample

Comparative analysis of antibiotic susceptibility profiles of clinical and environmental strains

A comparative study was carried out between the clinical and environmental samples isolated from salad samples, human sewage samples, chicken feces, and bovine samples. Results indicate strong differences in the numbers of different environmental isolates and the clinical strains in terms of the development of resistance to standard antibiotic disc.

B. cereus isolates collected from salad samples showed resistance to the antibiotic FOX30, PEF5 and F300 whereas *B. cereus* collected from human sewage sample showed resistance to PEF5, CIP5, and S10 when compared to clinical isolates of *B. cereus* for the same antimicrobial tested (Figure 3 and Figure 4). *S. flexneri* isolate collected from the feces of chicken showed resistance against two different antibiotics, FOX30 and E15, where as the susceptibility of their clinical strains to these antibiotics was moderately high. *E. coli* isolates collected from both salad and bovine samples showed resistance to two-three different antibiotics each (Figure 7 and Figure 8). On the other hand, the clinical strain of *E. coli* was considerably vulnerable to those same antibiotics. A noticeable fact in the susceptibility test result of *S. typhi* was that its clinical strain showed maximum level of susceptibility to the antibiotic C30, according to section 3.3, and one of its environmental isolates, namely H3 that was collected from human sewage samples showed resistance to this same antibiotic. In case of *Staphylococcus aureus*, its clinical strain was susceptible to all the antibiotics but one, and

yet its environmental isolates collected from both human sewage sample and chicken feces showed resistance to as many as four and eight different antibiotics (Annex 2).

Discussion

Antibiotic resistance is the ability of bacteria to endure the antimicrobial activity of antibiotics. It is now apparent that antibiotics that are used to alleviate an infection do not always work anymore. Antibiotic resistance is a global issue, and The US Centers for Disease Control and Prevention (CDC) considers antibiotic resistance as one of their peak concerns [7].

Antibiotics are, undoubtedly one of the greatest discoveries of the 20th century. This fact is evident, but the genuine speculation is the rise of antibiotic resistance in hospitals, communities, and the environment is associated with their use. The surprising yet alarming consequence is, the new genetic capacities of microorganisms have facilitated from man's overuse of antibiotics to exploit every source of their resistance genes and every means of horizontal gene transmission to develop multi-resistant strains [8, 9]. Such has been seen in this study, that the clinical strain of *Staphylococcus aureus* was significantly vulnerable to the antibiotics, yet the same strain isolated from the feces of chicken showed resistance to as many as eight different antibiotics. This is surely something to be concerned about.

Emergence of multidrug resistance has limited the therapeutic options, so monitoring the resistance pattern has vital importance [10]. Resistance has increasingly become an even bigger problem in recent years due to the drastically slowed pace at which novel antibiotics are being discovered, while antibiotic use is rising rapidly [11]. This study presents the most important aspects of antibiotic resistance development through a longitudinal study, with the conclusion that it is time to take action. To achieve complete reimbursement of therapeutic applications of antibiotics, we need to gather more information on the rise of antibiotic resistance. Creative approaches to the discovery of novel antibiotics and their accelerated and controlled introduction to therapy are mandatory, given the recent situation of antimicrobial resistance [12].

Antimicrobial resistance pattern monitoring will help us to review the current status of antimicrobial resistance locally, nationally and globally minimize the consequence of drug resistance and limit the emergence and spread of drug resistant pathogens. This has been a major endeavor of this study. Resistance to antibiotics is increasing and imperative community health problems are at risk. An accelerated start up for developing new antibiotics and taking measures to conserve the existing microbial agents can be our way to alleviate the current problem. Also the widespread usage of antibiotics should be brought to a proscribed manner along with the measures to help control the bacterial spread to slow down the emergence and spread of resistant organisms.

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Annexure 1

Table 3: Antibiotic susceptibility test results for clinical strains

Antibiotics	Clinical Strains				
	S. typhi	***E. coli* K12**	***B. cereus***	***S. flexneri***	***S. aureus***
SXT 25	33 mm	30 mm	9 mm	0 mm	28 mm
FOX 30	32 mm	28 mm	24 mm	26 mm	30 mm
PEF 5	22 mm	32 mm	25 mm	0 mm	32 mm
CIP 5	27 mm	40 mm	28 mm	16 mm	28 mm
E 15	8 mm	9 mm	28 mm	20 mm	9 mm
CN 10	25 mm	20 mm	25 mm	22 mm	20 mm
K 30	25 mm	19 mm	24 mm	24 mm	20 mm
S 10	17 mm	14 mm	20 mm	0 mm	14 mm
CXM 30	26 mm	25 mm	0 mm	26 mm	26 mm
NA 30	0 mm	24 mm	0 mm	0 mm	25 mm
OX 1	0 mm	0 mm	0 mm	0 mm	0 mm
C 30	34 mm	24 mm	26 mm	16 mm	26 mm
F 300	23 mm	26 mm	22 mm	24 mm	24 mm

Annexure 2

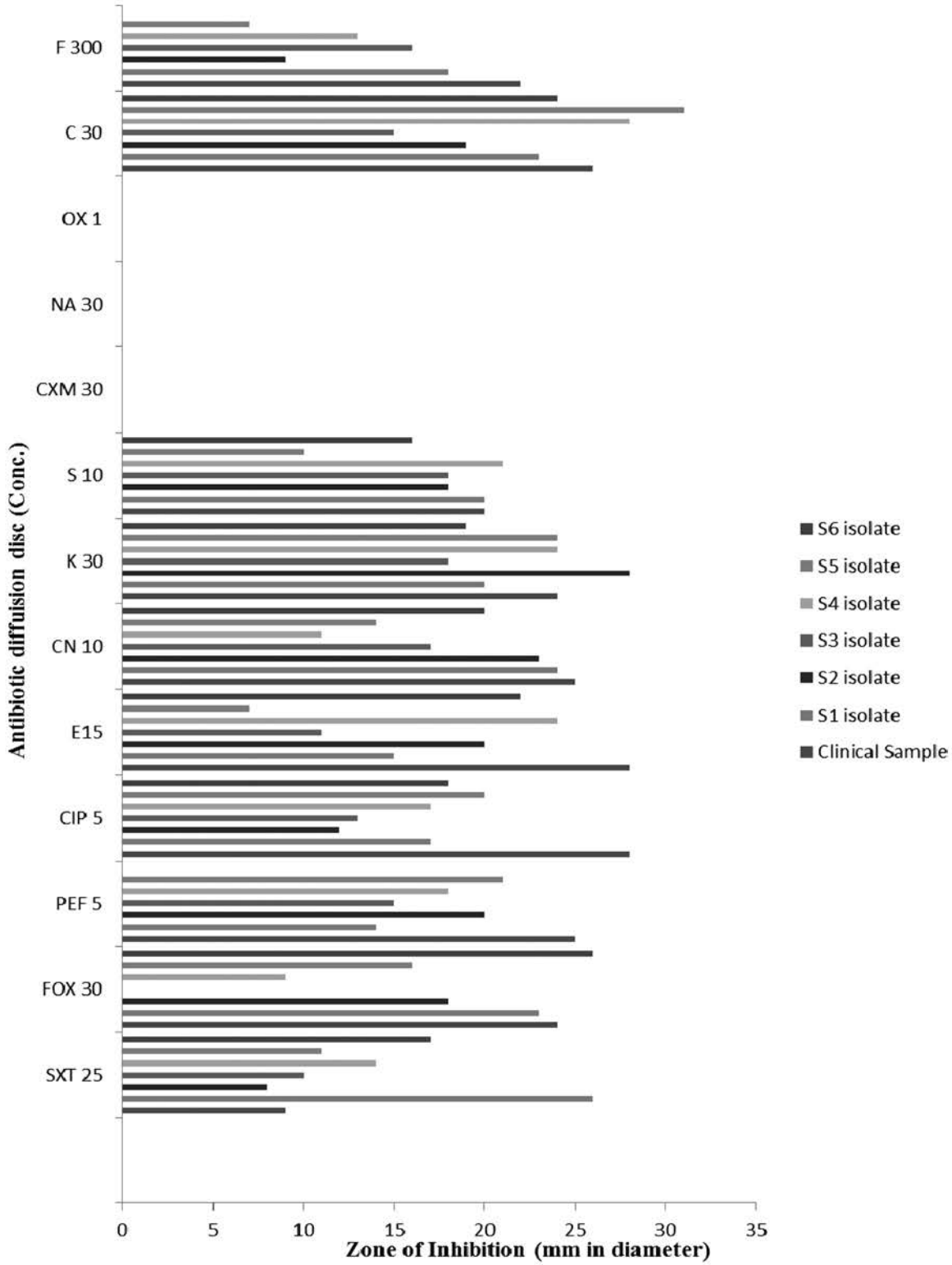

Fig. 3: Comparison among the efficiency of different antibiotics on clinical and environmental samples of B. cereus spp collected from salad sample

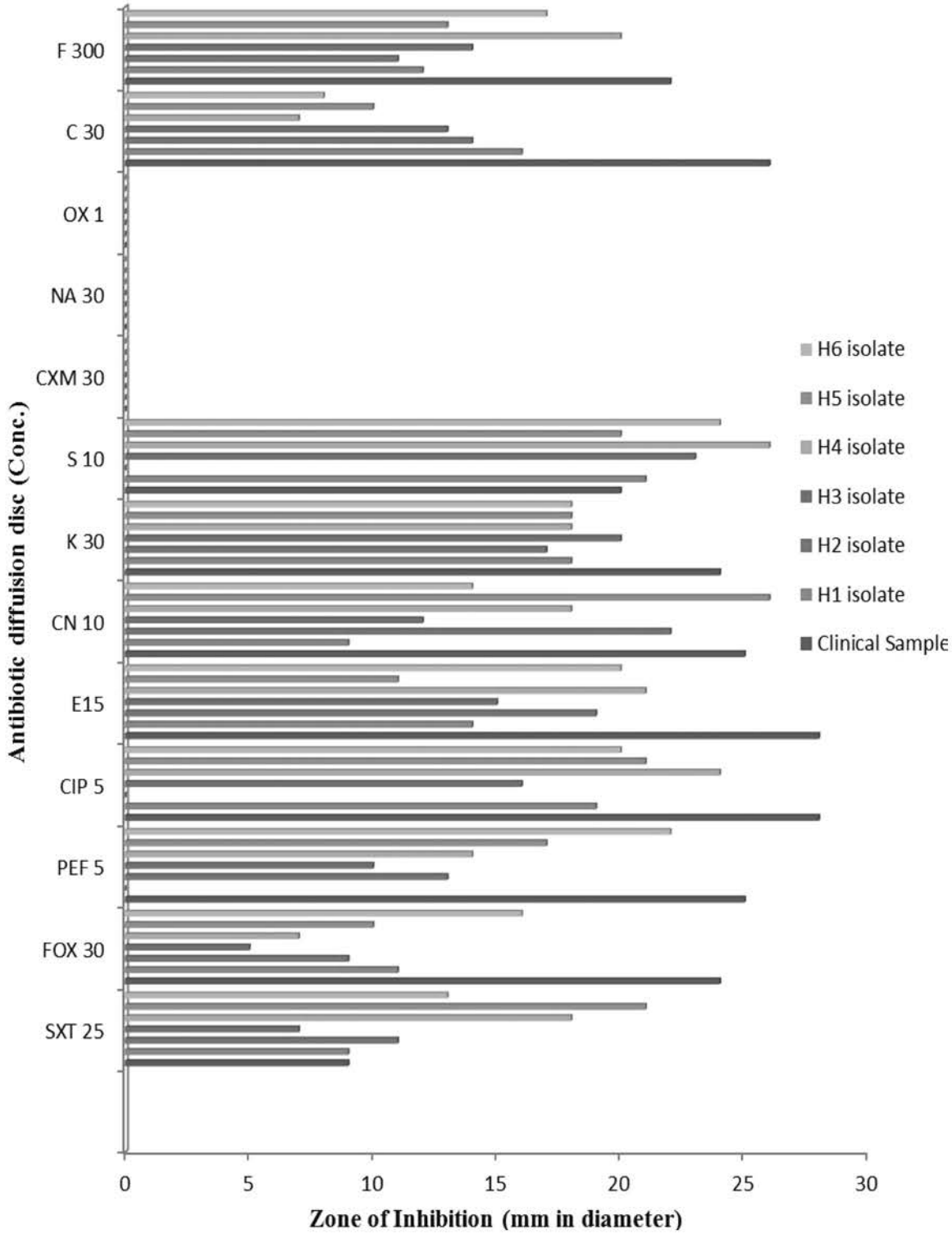

Fig. 4: Comparison among the efficiency of different standard antibiotics on clinical and environmental samples of B. cereus spp collected from human sewage sample

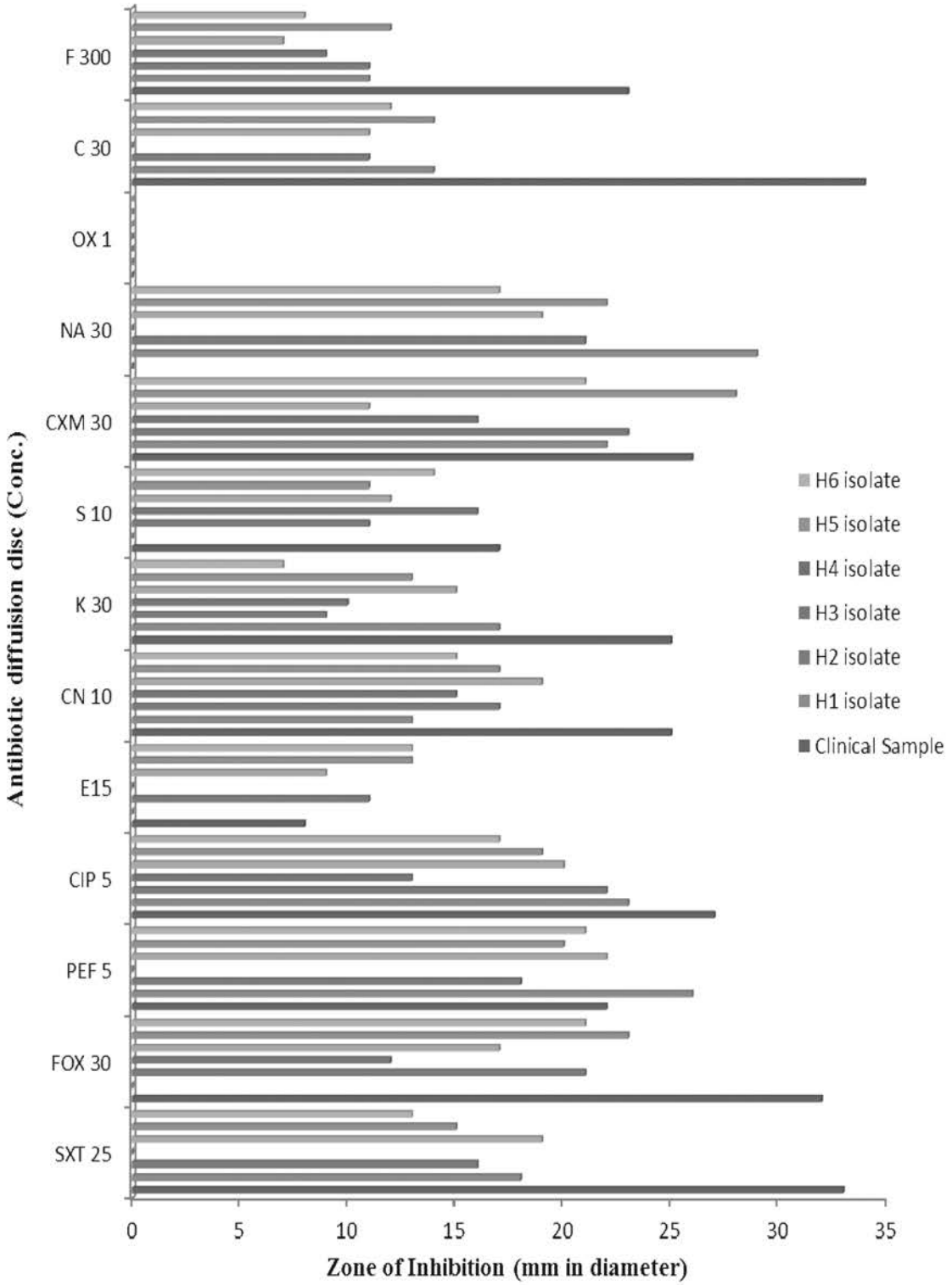

Fig. 5: Comparative analysis of the effect of different antibiotics on clinical and environmental Salmonella typhi spp collected from human sewage sample

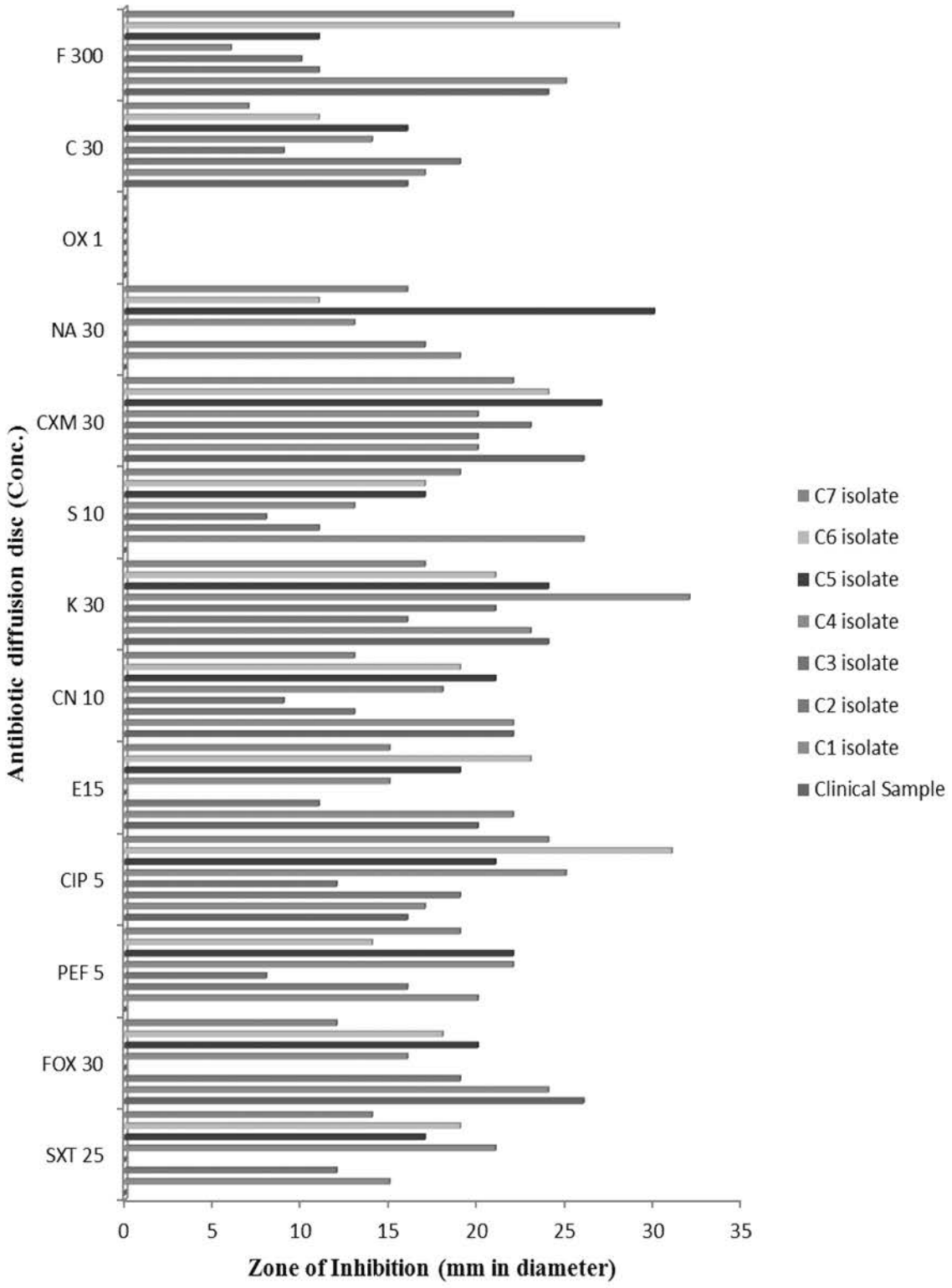

Fig. 6: Comparative analysis of the effect of different antibiotics on clinical and environmental Shigella flexneri spp collected from chicken feces

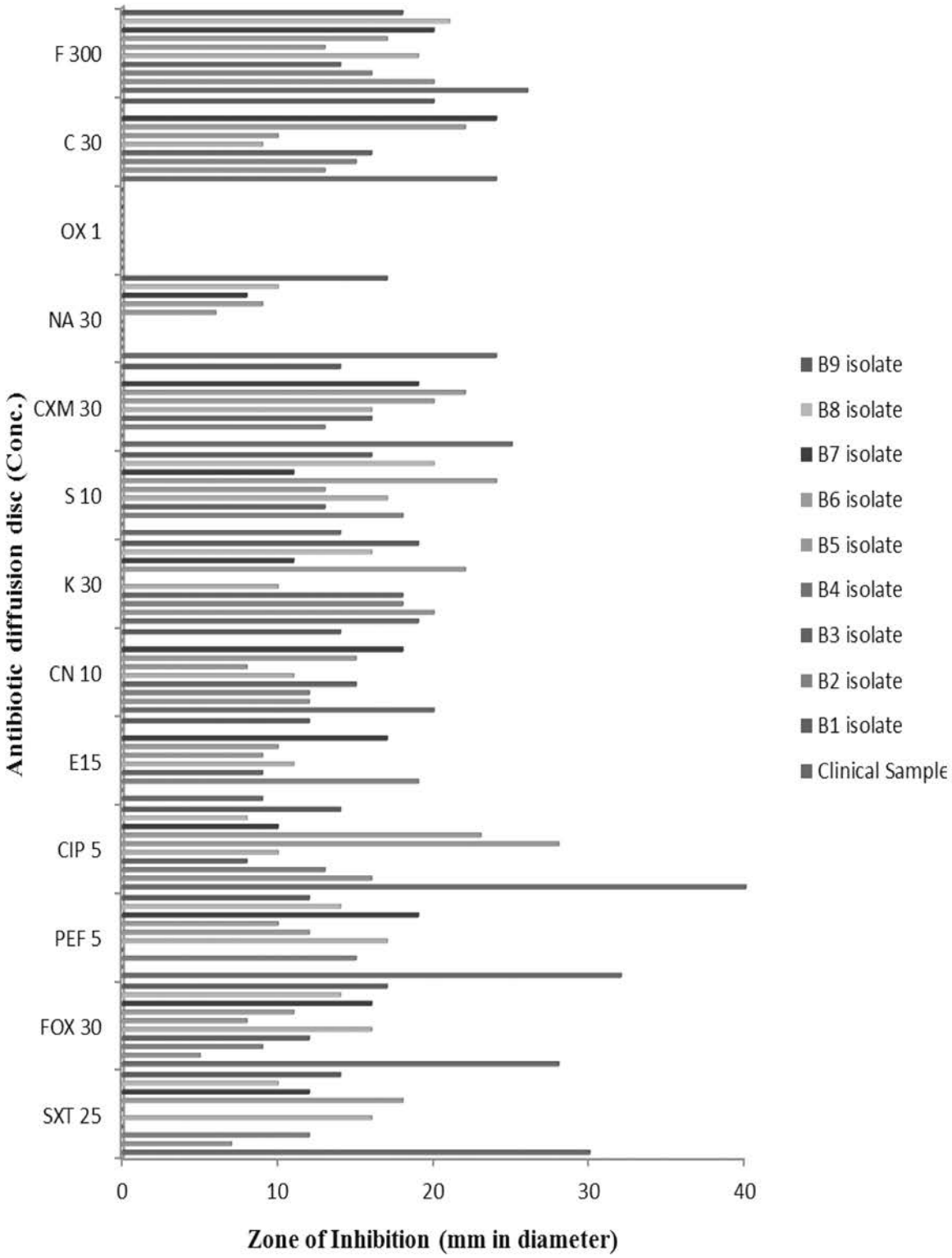

Fig. 7: Comparison among the efficiency of different antibiotics on clinical and environmental samples of E. coli spp collected from bovine sample

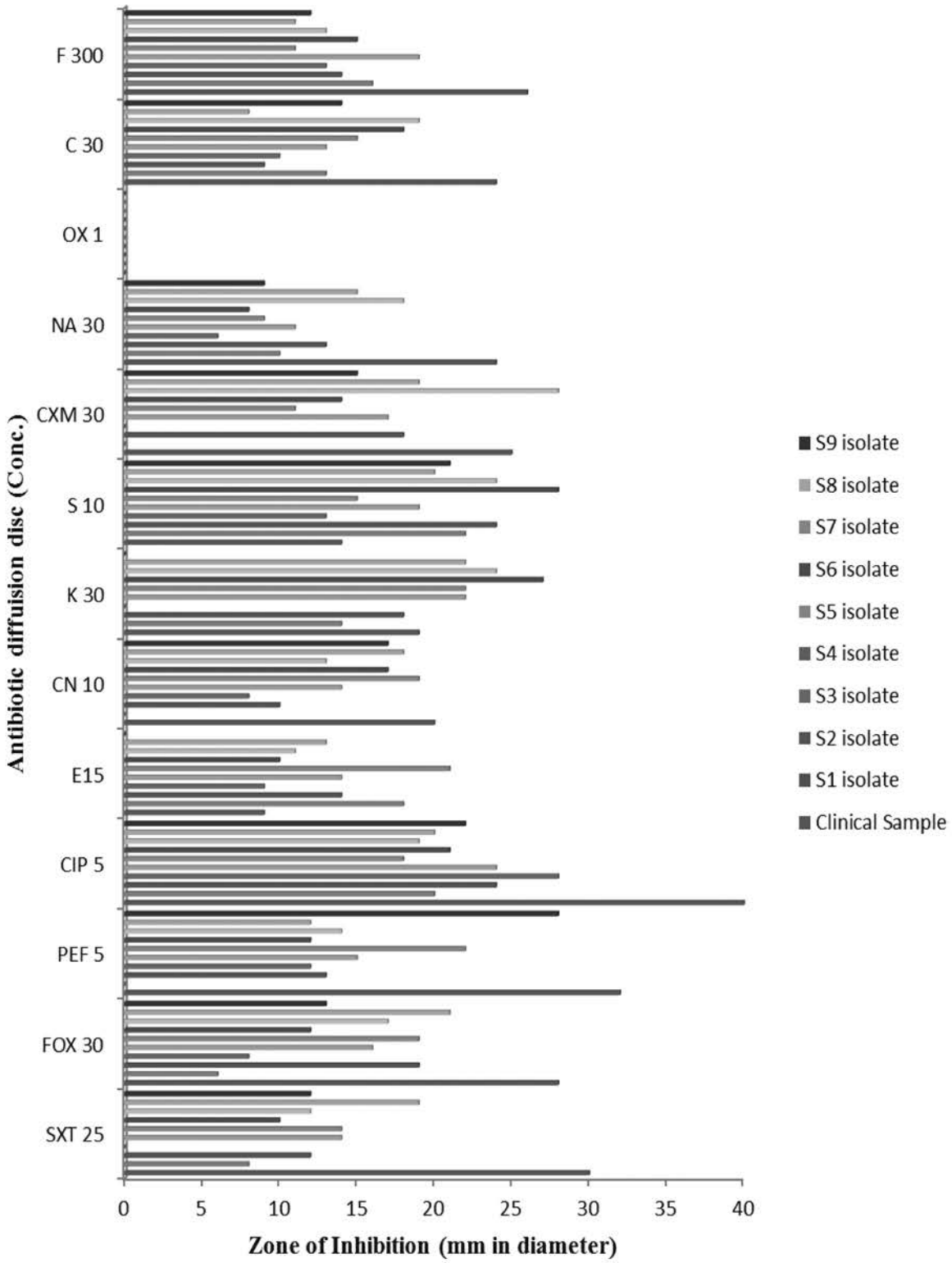

Fig. 8: Comparison among the efficiency of different antibiotics on clinical and environmental samples of E. coli spp collected from salad sample

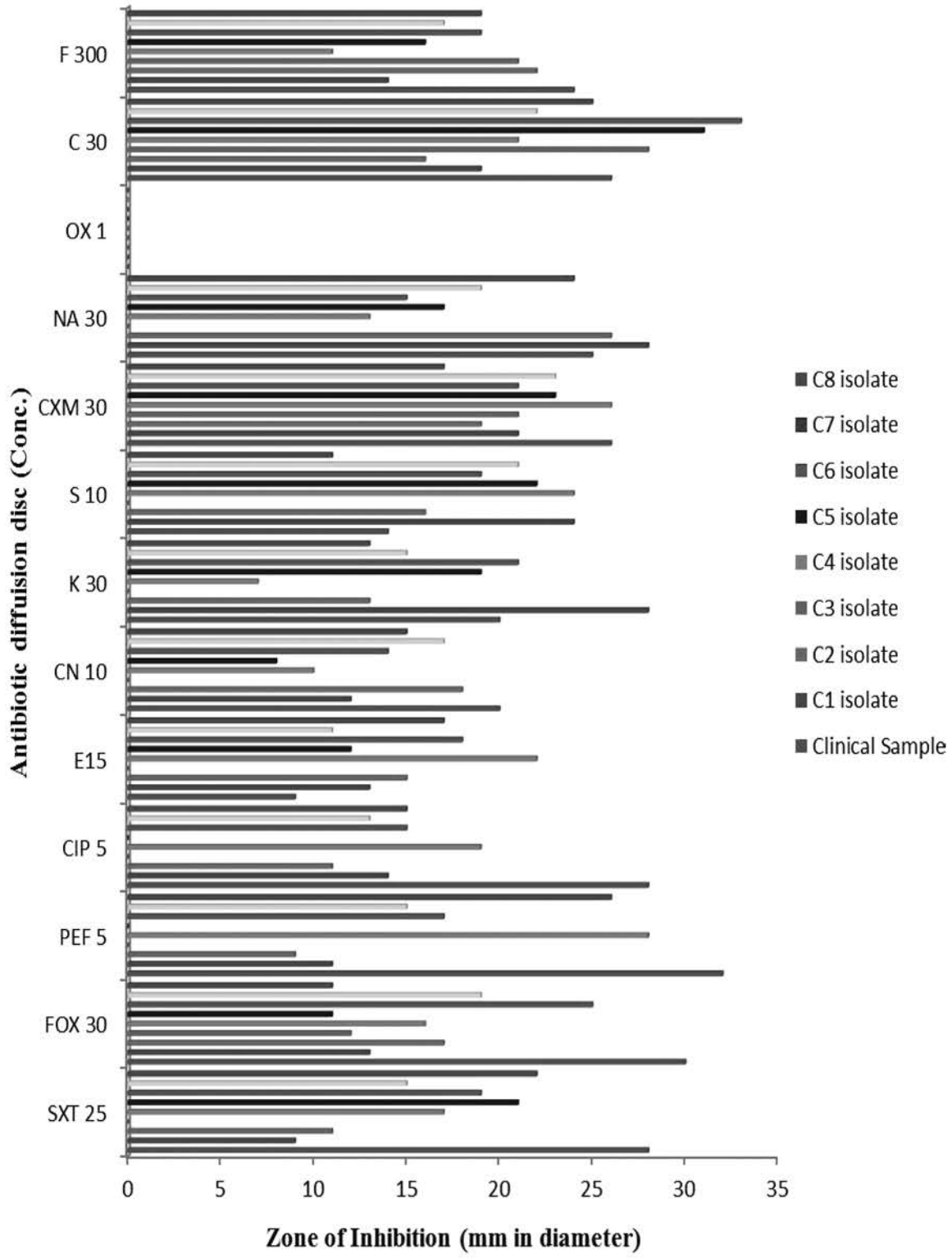

Fig. 9: Comparison among the efficiency of different antibiotics on clinical and environmental samples of Staphylococcus aureus spp collected from chicken feces

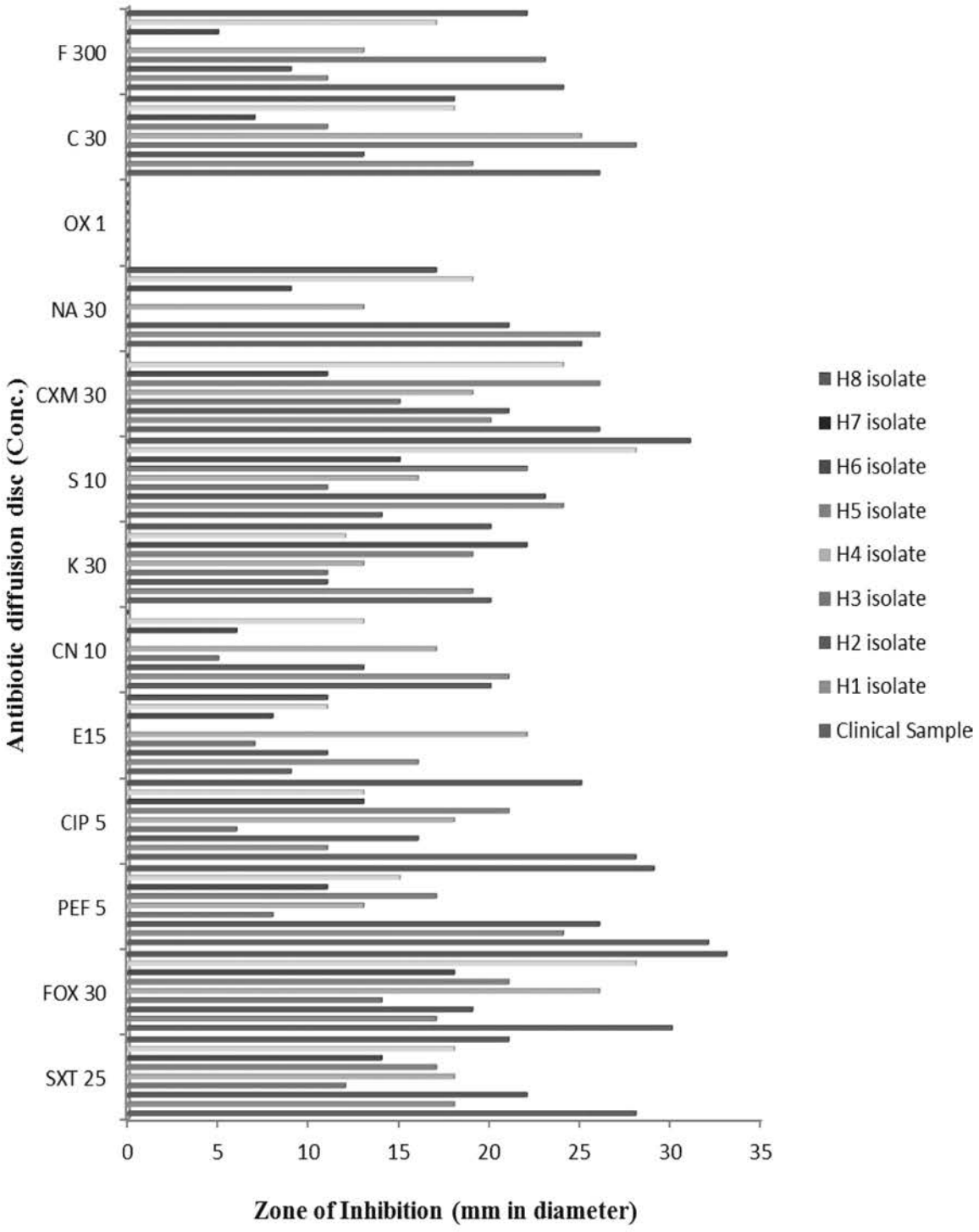

Fig. 10: Comparison among the efficiency of different antibiotics on clinical and environmental samples of Staphylococcus aureus spp collected from human sewage sample