



The occurrence of ESBL Producing *Escherichia coli* and *Klebsiella* species in Selected Broiler Farms in Jalingo, Taraba State Nigeria

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ABSTRACT

Food-producing animals, including poultry, have been considered as potential sources of extended-spectrum β -lactamase (ESBL) - producing *Escherichia coli*. This study investigates the occurrence and distribution of ESBL-producing *E. coli* and *Klebsiella* species among three broiler poultry farms in Jalingo, Taraba State Nigeria. One hundred and thirty-eight isolates which comprised of *E. coli*, *K. pneumoniae* and *K. oxytoca* were isolated and successfully identified from the broilers farms. The isolates were tested for beta-lactamase producing ability as well as screened for the antibiotic susceptibility profile and the curing rate of the ESBL positive bacteria using standard procedures. While the results obtained revealed the occurrence of 94.90%, 3.00% and 2.20% for the *E. coli*, *K. pneumoniae* and *K. oxytoca* in the farms respectively, the prevalence of ESBL producing bacteria isolates was 34.10%, 0.70% and 0% respectively for the *E. coli*, *K. pneumoniae* and *K. oxytoca*. It was also revealed that clavulanic acid inhibited the ESBL enzyme. The antibiotic susceptibility profile of the ESBL producing *E. coli* showed a 100% resistance to ampicillin, ceftazidime and ceftriaxone as well as a 46.80%, 70.20%, 2.10%, 25.50% and 59.60% resistance to chloramphenicol, ciprofloxacin, nitrofurantoin, gentamicin and tetracycline respectively. The curing rate of the ESBL positive producing *E. coli* was 16.70%. This study revealed the presence of ESBL-producing *E. coli* in chickens from small-scale commercial poultry farms in Jalingo, thus indicating that chickens may serve as important reservoirs for the transmission of antimicrobial-resistant pathogens to humans through the food chain.

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INTRODUCTION

A high percentage of hospital-acquired infections are caused by drug-resistant bacteria such as extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* (Paterson and Bonomo 2005). Patients with

infections caused by drug-resistant bacteria are generally at high risk of worse clinical outcomes and death and hence require more healthcare resources than patients infected with the same bacteria that are not resistant (Tumbarello, 2006).



The evolution of resistant strains is a natural phenomenon that occurs when microorganism themselves erroneously mutate or when resistant traits are exchanged between them. The use and misuse of drugs accelerate the emergence of drug-resistant strains (Cheesbrough, 2010). Poor infection control and inappropriate food-handling encourage the further spread of antimicrobial resistance. New resistance mechanisms emerge and spread globally threatening our ability to treat common infectious diseases, resulting in the death and disability of individuals (Tumbarello, 2006). The achievements of modern medicine are put at risk by antimicrobial resistance. Without effective antimicrobial agents for the prevention and treatment of infectious diseases, the success of organ transplantation, cancer chemotherapy and major surgery would be compromised (WHO, 2014).

ESBL producing bacteria destroy the beta-lactam ring of penicillins and cephalosporins thereby rendering the antimicrobials inactive. ESBLs hydrolyzes oxyimino-cephalosporins such as cefuroxime, cefotaxime, ceftriaxomine, ceftizoxime, ceftazidime, celpirome and cefepime, aztreonam as well as penicillins and another cephalosporin except for cephamycin (cefoxitin and ceftetean) (Susic, 2004). ESBL producing gram-negative organisms limit the therapeutic options as well as their multidrug resistance (Anago *et al.*, 2015). Drug resistance in animals is caused mainly by the large number of antimicrobial drugs used in food production. Grave *et al.*, (2010) reported that the Netherlands is one of the highest users of antimicrobial agents in food production for animals which results in a high rate of drug resistance. This study was therefore aimed at evaluating the occurrence of ESBL producing *E.coli* and *Klesiella* species isolated from selected broilers' farms in Jalingo, Taraba State.

MATERIALS AND METHODS

Study Area

Jalingo Local Government Area, the capital of Taraba State located in the North-East geopolitical zone and situated between latitude 8° 47' North and 90°1' North and longitude 11°9' East and 11°30' East was the study location.

Sample Collection

Samples were collected from three poultry farms which comprised of 2 broiler chicken farms with chickens 7 weeks old and one broiler chicken farm with chickens more than 7weeks old. The broilers chickens used for this study were all healthy and bred specifically for meat production. The samples were cloaca, stool and floor swabs. Swab sticks were soaked in sterile distilled water and inserted into the cloaca of randomly selected chickens in the selected poultry farms. While in the cloaca, the swab stick was rotated three times before inserting it into its container. Sterile distilled water swab sticks were used to swab strategic areas on the floor of the poultry. A wide-mouthed sterile container was used to collect the stool of each of the chicken rearers.

Bacteria Isolation Procedures and Identification

Samples collected were cultured within 2 hours of collection on MacConkay agar and Eosin methylene blue (EMB) agar (Oxoid CM 516, UK) before being incubated at 37°C for 18–24 hours. Distinct colonies were examined for their morphology and ability to ferment lactose. Lactose fermenting colonies were further subcultured on the EMB agar, incubated for 18 h at 37°C and then examined for the appearance of metallic greenish colonies. All isolates (both lactose and non-lactose fermenters) were individually gram stained and subjected to biochemical tests. DNA sequencing using the sangar sequencing



technique was used for the molecular characterization of the isolates

ESBL Detection

Isolates were tested for beta-lactamase production using the paper acidimetric method as previously outlined by Cheesbrough, 2010. All the positive β -lactamase isolates were screened for ESBL production by double-disk synergy test (DDST) according to the procedures outlined by Liofichem (2014). Briefly, four millilitres of 0.5 Mc Farland equivalent standard of the test organisms were spread on the surface of a sterile Mueller Hinton agar plate using a sterile swab stick. After 20 minutes, the combination of augmentin disc of concentration 30 μ g, amoxicillin of concentration 20 μ g and clavulanic acid of concentration 10 μ g was placed 15 mm apart from the centre of ceftriaxone disc of 30 μ g concentration and ceftazidime disc of concentration 30 μ g. This was incubated for 18 h at 37°C.

Antimicrobial Susceptibility Testing

The modified Kirby-Bauer disc diffusion method as described by the Clinical and Laboratory Standards Institute guidelines was adopted (CLSI, 2012). Briefly, suspensions were made from the 24 h growth of the organism maintained in saline to match the 0.5 McFarland turbidity standards. This was spread on the entire surface of Mueller-Hinton agar plates containing ceftazidime (30 μ g), ceftriaxone (30 μ g), ampicillin (10 μ g), nitrofurantoin (300 μ g), gentamicin (10 μ g), chloramphenicol (30 μ g), tetracycline (30 μ g) and ciprofloxacin (5 μ g) using a sterile swab stick. The agar plates were incubated at 35°C for 18–24 h, after which the diameter of the zones of growth inhibition around the discs was measured using a measuring ruler. The results were further

interpreted using the Performance Standards for Antimicrobial Susceptibility Testing (CLSI, 2012).

Plasmid Profile of ESBL Positive Isolates

The plasmid profile of the ESBL positive isolates was investigated according to the modified alkaline lysis plasmid extraction protocols described by Dagan *et al.* (2006) and Charles *et al.* (2010) respectively. A 1% Agarose gel was prepared and loaded into an electrophoresis chamber containing 8 wells; buffered with 40 mM Tris, 20 mM, 2 mM EDTA and adjusted to pH 7.8 with acetic acid. Electrophoresis was allowed to proceed at room temperature for 20 min after which the gels were stained with ethidium bromide (1 μ L/mL) and viewed under UV transillumination. The molecular marker that was used was the *Hind Pfl III* digest and extrapolations were made from the Electropherogram obtained (Piglansky *et al.*, 2003).

Plasmid Curing

ESBL positive isolates were selected and subjected to acridine orange as described by Stanisich (1988). Each tested organism was grown in a solution of 5ml double strength nutrient broth supplemented with 0.1 ng/ml acridine orange and incubated at 37°C for 24 h. After incubation, the test organisms were retested for ESBL production using the double-disk synergy test (DDST).

RESULTS AND DISCUSSION

The result of the 138 microbial isolates obtained from the 3 broiler chicken farms shows that one hundred and thirty-one were *E. coli*, four were *K. pneumoniae* and three were *K. oxytoca* constituting 94.90%, 2.90% and 2.20% respectively. This result is presented in Figures 1 (a) – (c).

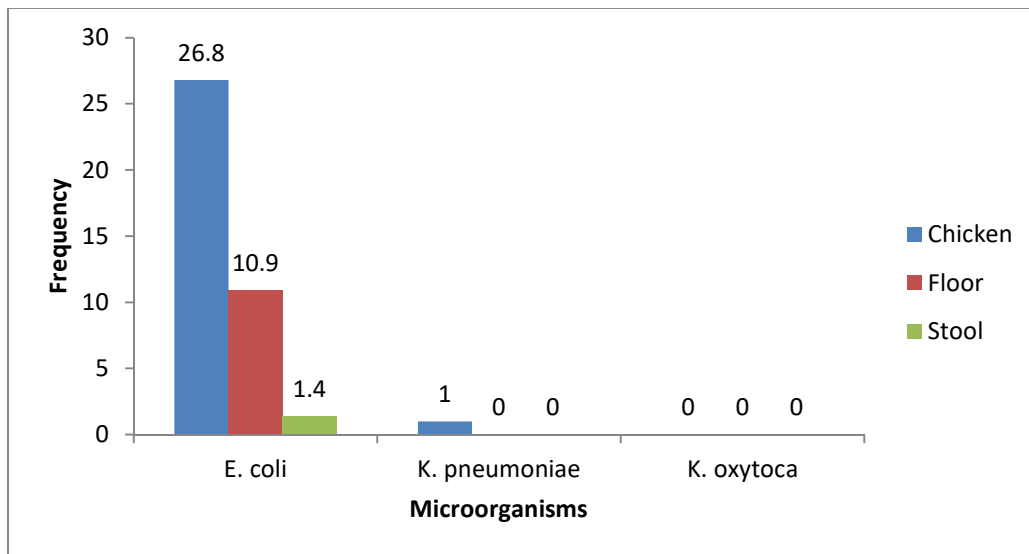


Figure 1 (a): Distribution of Bacterial Isolates obtained from the chicken cloacae, rearer stool and floor from the farm I containing 7 weeks old broiler chickens

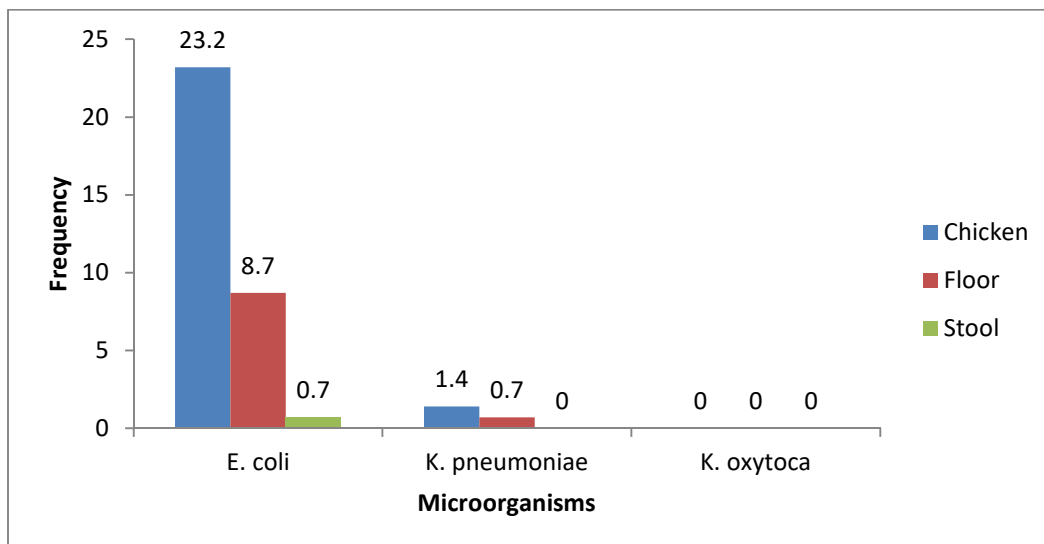


Figure 1 (b): Distribution of Bacterial Isolates obtained from the chicken cloacae, rearer stool and floor from farm II containing 7 weeks old broiler chickens

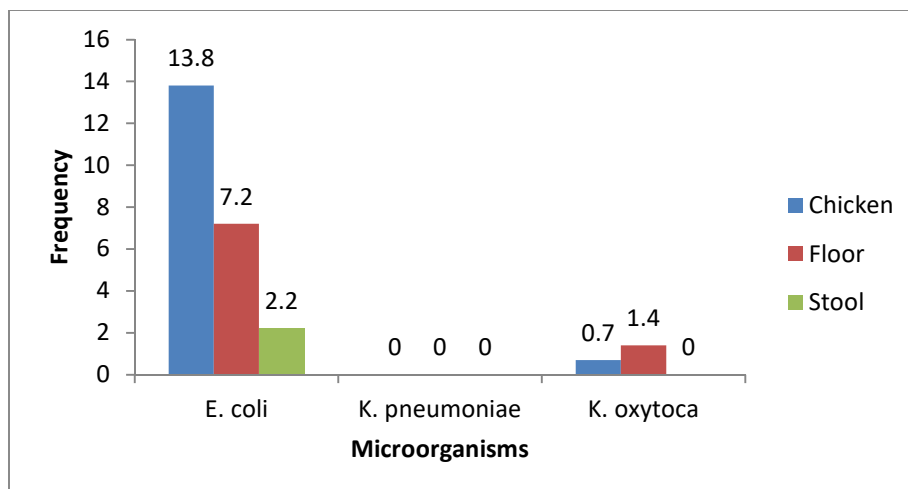


Figure 1 (c): Distribution of Bacterial Isolates obtained from the chicken cloacae, rearer stool and floor from farm III containing over 7 weeks old broiler chickens

The number of the isolated bacteria was higher in the cloaca and stools of the chicken in all the farms investigated compared to the rearers stool and pen floors because the cloaca swabs and the stools were the contaminated samples with the floors merely contaminated as a result of the chicken's droppings. The high occurrence of *E. coli* (94.9%) obtained in this study is higher than the 44% reported by Ashraf *et al.* (2015) upon an investigation of the prevalence of *E. coli* on imported chickens. Nwakaeze *et al.* (2013) and Dadheech *et al.* (2016) also reported isolated 70.50% and 100% respectively of *E.*

coli isolates from faecal and cloacal swab samples. The 3.0% *K. pneumoniae* occurrence rate in this study is lower than the 5.8% reported by Hassan *et al.* (2015). A literature search by the researchers revealed that there has never been any report of the occurrence of *K. oxytoca* in chickens.

The result of the synergy between the clavulanic acid-containing disk with ceftazidime and ceftriaxone in the Double Disk Synergy Test (DDST) as well as the frequency exhibited by the ESBL positive *E. coli* among the 138 microbial isolates is presented in plate 1 and figure 2 (a – c).



Plate 1: Disc showing the synergy exhibited by the ESBL positive *E.coli* of clavulanic acid with ceftazidime and ceftriaxone in the Double Disk Synergy Test. Key: 1 - ceftazidime; 2 - ceftriaxone; 4 – augmentin.

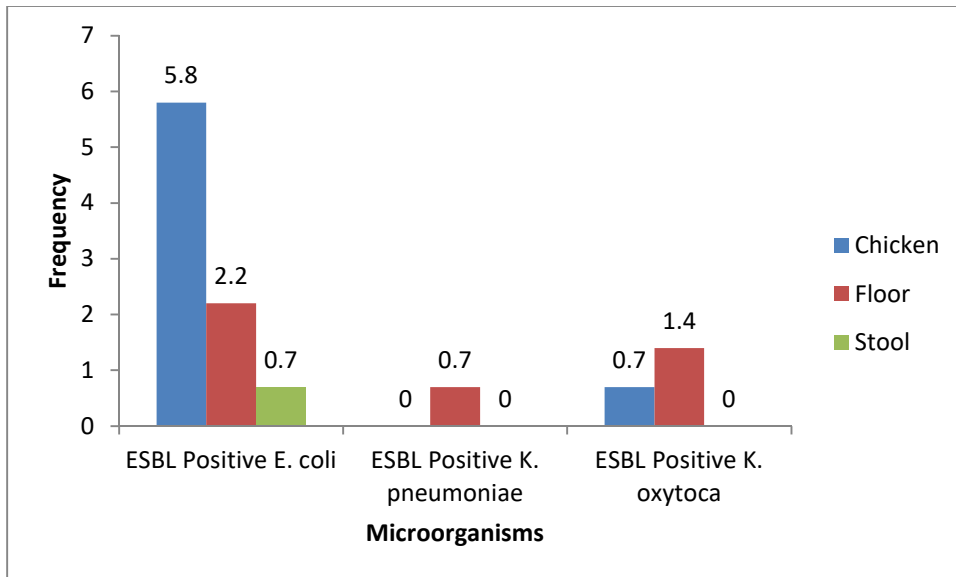


Figure 2 (a): Distribution of ESBL positive microbial isolates obtained from the chicken cloacae, rearer stool and floor from the farm I containing 7 weeks old broiler chickens

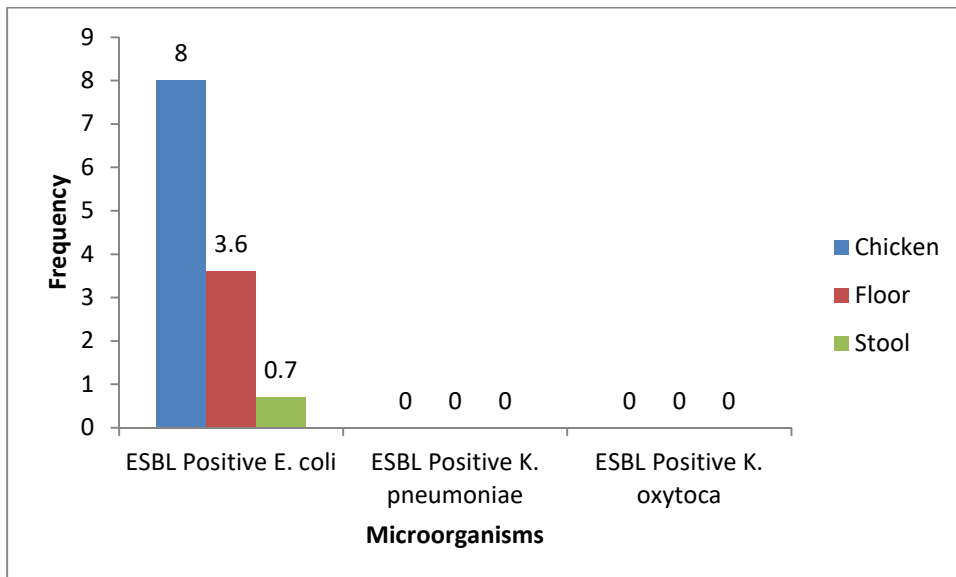
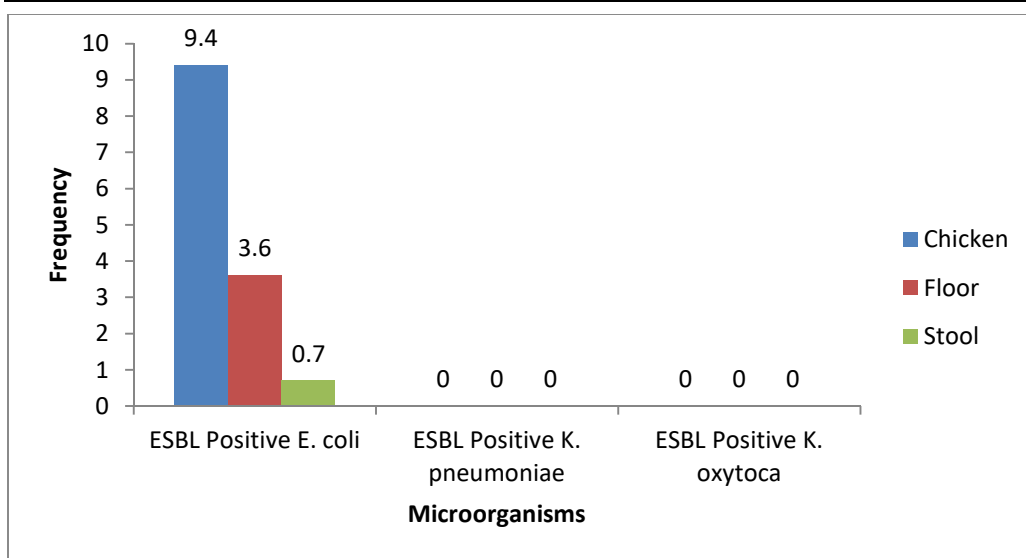


Figure 2 (b): Distribution of ESBL positive microbial isolates obtained from the chicken cloacae, rearer stool and floor from farm II containing 7 weeks old broiler chickens



Figures 2 (c): Distribution of ESBL positive microbial isolates obtained from the chicken cloacae, rearer stool and floor from farm III containing over 7 weeks old broiler chickens

The result obtained from the DDST test showed that the zone of growth inhibition around ceftazidime (disc 1) and ceftriaxone (disc2) augmented towards the antibiotic disc 4 which contained clavulanic acid (augmentin). This is because clavulanic acid inhibited the action of ESBL. The synergy of clavulanic acid with third-generation cephalosporin was previously reported by Oyinloye and Ezekiel (2011). The occurrence rates of ESBL producing *E. coli*, *K. pneumoniae* and *K. oxytoca* as reported in figures 2 (a-c) showed that while was *E. coli* has a prevalence rate of

34.1% higher than 14.2% reported by Nwakaeze *et al.* (2013) with *K. pneumoniae* having a prevalence rate of 0.7% in agreement with the findings of Hiroi *et al.* (2012) who isolated one ESBL producing *K. pneumoniae* from the faecal samples of broilers. No occurrence was however observed with the *K. oxytoca* bacteria. The susceptibility profile of the ESBL positive producing *E. coli* of the microbial isolates as well as their curing rate when challenged with acridine orange is presented in tables 1 as 2 as presented below.

Table 1: Antibiotic susceptibility profile of the ESBL positive *E. coli*

Antibiotics	(µg/disc)	S	%	I	%	R	%
Ampicillin	10	0	0	0	0	47	100
Chloramphenicol	30	14	29.8	11	23.4	22	46.8
Ciprofloxacin	5	12	25.5	2	4.3	33	70.2
Nitrofurantoin	300	42	89.4	4	8.5	1	2.1
Gentamicin	10	32	60.1	3	14.4	12	25.5
Tetracycline	30	11	23.4	8	17	28	59.6
Ceftriaxone	30	0	0	0	0	47	100
Ceftazidime	30	0	0	0	0	47	100

Key: S – sensitive; I – Intermediate; R – Resistant; % - Percentage



Table 2: Curing rate of the ESBL positive *E. coli* isolates

Farm	Bacteria	No of ESBL +ve Isolates	No of Isolates without plasmid after curing	% of isolates cured
I	<i>E. coli</i>	11	1	2.1
	<i>K. pneumoniae</i>	1	0	0
	<i>K. oxytoca</i>	0	0	0
II	<i>E. coli</i>	17	3	6.3
	<i>K. pneumoniae</i>	0	0	0
	<i>K. oxytoca</i>	0	0	0
III	<i>E. coli</i>	19	4	8.3
	<i>K. pneumoniae</i>	0	0	0
	<i>K. oxytoca</i>	0	0	0

Key: No – Number; ESBL – Extended Spectrum Beta Lactamase; +ve – Positive; % - Percentage

The antibiotic susceptibility profile of ESBL positive *E. coli* presented in table 1 revealed that the bacteria showed 100% resistant to ampicillin, ceftriaxone and ceftazidime. The isolates were however 46.80%, 70.20%, 2.10%, 25.50% and 59.60% resistant to chloramphenicol, ciprofloxacin, nitrofurantoin, gentamicin and tetracycline respectively. The multidrug resistance observed in this study agrees with the report by Motayo *et al.* (2013) and Afunwa *et al.* (2011).

The curing rate of the ESBL positive isolates revealed that 16.70% of the ESBL positive isolates were cured while 69.20% were not (Table 2). This supports previous reports by Iroha *et al.* (2010) and Folasage *et al.* (2014).

CONCLUSION

The findings from this study confirm broilers' farms as reservoirs of ESBL producing *E. coli* and *K. pneumoniae*. The occurrence of ESBL producing *E. coli* was higher than that reported for *K. pneumoniae*.

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