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Public health implication of the detection of pathogenic bacteria in beef during processing in abattoirs from Benin City, Nigeria

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SUMMARY. The aim of this study was to determine the presence of pathogenic bacteria in beef during processing in abattoirs within Benin City, Nigeria. A total of 100 samples were obtained from12 sales tables and 8 processing halls during the study period. Isolation, enumeration and characterization of bacterial isolates were carried out using standard methods. Antibiogram of the test isolates was determined using disc diffusion technique. Bacterial isolates were screened for virulence genes. The results of this study showed that the highest total bacterial count was recorded in the processing hall at abattoir 4 ($9.28 \pm 0.26 \times 10^3$ cfu/cm^2) and the least ($3.47 \pm 0.19 \times 10^3$ cfu/cm^2) was from the processing hall at abattoir 2. The identified isolates were *Escherichia coli, Staphylococcus* sp. and *Salmonella* sp. All were multi-drug resistant. In this study, 11 *Escherichia coli* isolates were screened for the *tsh* (temperature sensitive haemagglutinin) virulence gene and 63.6% were positive for the *tsh* virulence gene. The virulence-associated gene in *Staphylococcus* sp. showed that only 22.2% tested positive to *hlg* (gamma hemolysin) gene while 93.3% of *Salmonella* sp. were positive for the *inv*A (invasive protein) gene. These results revealed the presence of multi-drug resistant bacterial isolates with virulence properties in beef during processing in abattoirs. Therefore, strict hygiene measures should to be put in place to combat the proliferation of these pathogenic bacterial isolates. In addition, misuse and abuse of antibiotics should be prohibited as these pathogens are becoming more resistant to most conventional drugs, thereby making associated diseases difficult to cure.

Keywords: abattoir, antibiotics, bacteria, pathogens, virulence genes

Introduction

Food safety is a complex issue, whereby animal proteins such as meats and meat products are generally regarded as a high risk commodities, to infection and toxicity (Yousef *et al.,* 2008). Diseases arising from ingestion of bacteria, toxins and also cells produced by microorganisms present in food are referred to as food borne illnesses (Clarence *et al.,* 2009).

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Meat and meat products are sometimes contaminated with germs in the abattoirs as a result of the use of cotaminated equipment, during handling and power shortage during storage as power outage results in the reduction of animal products' shelflife (Stagniitta *et al.,* 2006). Hygiene conditions are poor when foods are produced in non-industrial establishments, mainly due to insufficient monitoring or improper conditionsduring processing. These contaminated food ends up infecting or intoxicating children, elderly and immuno-suppressed individuals who are highly susceptible (Stagnitta *et al*., 2006). Raw beef and beef products could inevitably contain pathogenic microorganisms (Nichlos and de Louvous, 1995). Various Gram-negative *(Escherichia* sp, *Enterobacter* sp, *Yersinia* sp, *Pseudomonas* sp.and *Salmonella* sp.) and Gram-positive bacteria (Bacillus sp., Micrococcus sp. and Lactobacillus sp.) are frequently isolated from the meat surface (Polster and Hartiova, 1985). Gracey (1981) reported that, the organisms responsible for food poisoning by infection were *Salmonellae, Escherichia coli* and *Vibrio parahaemolyticus*. Those responsible for poisoning by toxin production included *Staphylococcus aureus, Clostridium perfringens, Clostridium botulinum, Bacillus cereus* and Streptococci. Other bacteria which may cause occasionally outbreaks of food poisoning included: *Streptococci, Proteus, Pseudomonas, Providencia, Citrobacter, Aeromanas Hydrophila, Yersinia enteracolitica, Campylobacter,* and *Shigella flexineri* and are the most commonly implicated organisms in food-borne illnesses (Meadand Dietz, 1999).

Many of the slaughter houses/abattoirs are more than 50 years old without adequate basic amenities viz. proper flooring, ventilation, water supply, lairage, transport etc. In addition to these deficiencies, slaughter houses/abattoirs suffer from very low hygiene standard posing a major public health and environmental hazards due to discrete disposal of waste and highly polluted effluent discharge. Bacterial infections/diseases are widely treated with a variety of antibiotics in both animals and humans (Erb *et al*., 2007). However, misuse of antibiotics in clinical *and veterinary settings has resulted in the emergence of multidrug-resistant* microbes (Schierack *et al*., 2006; Wang *et al*., 2011). Researchers have characterized that *antibiotic resistance is more common in pathogens compared to commensal* organisms, and is linked to the association between resistance and virulence factors or due to frequent exposure of pathogenic strains to antibiotics (Boerlin *et al*., 2005).

A subset of genes are key players in the ability of a bacterium to cause disease. The products of such genes facilitate the successful colonization and survival of the bacterium in or cause damage to the host (Coulter *et al*., 1998). Bacterial virulence factors may be encoded on the chromosomal DNA, plasmid, transposon or temperate bacteriophage DNA. Other virulence factors are acquired by bacteria following infection by a particular bacteriophage, which integrates its genome into the bacterial chromosome by the process of lysogeny. The virulence factors of bacteria can be divided into a number of functional types, these are 1) The adherence and colonization factors, 2) The invasion factors, 3) Capsules and other surface components, 4) Endotoxins and 5) Exotoxins (Peterson, 1996). This ability of bacteria establishes the pathogenic success of well-adapted gastrointestinal pathogens such as that differentially coordinate the expression of sets of genes as they pass from one host environment to another in their passage through the gut, including the movement through the gastric barrier and survival within macrophages or intestinal epithelial cells (Chaudhuri *et al*., 2013).

The aim of this study was to determine the presence of pathogenic bacterial isolates in abattoirs during processing. The objectives were to:

i. determine the bacterial count in the sales tables and processing halls.
ii. isolate and characterize the bacterial isolates
iii. determine the antibiotic susceptibility profile of the isolates.
iv. determine the presence of virulence genes in the isolates.

Materials and methods

Study site

A total of four (4)abattoirs in Benin City, Edo State, Nigeria were used for this study. Samples were collected from cow skin, hands of handlers, processing tables and floors. The cows were kept in the lariages before they were slaughtered and afterwards stored in cold rooms.

Sample collection

Samples were collected by swabbing a 100 cm^2 area of the sales tables and floor of processing halls with sterile swab sticks which were pre-moistened with 2 % W/V peptone water. After swabbing, the swab sticks were put into a sterile containers and stored in ice while being transported to the laboratory (ISO 18593, 2004).

Isolation and enumeration of bacteria

All samples were cultured by the pour-plate method on Nutrient agar for total bacterial count. Plates were incubated at 37 ^oC for 24 hours, after which the colonies grown were counted using standard plate count method (ISO 18593, 2004).

Characterization of Isolates

Samples were plated on MacConkey agar, Mannitol salt agar and Xylose Lysin Deoxycholate agar using the spread plate method. This was followed by aerobic incubation at 37 ^oC for 24 hours. Discrete pinkish colonies on the MacConkey agar were isolated and sub-cultured to obtain pure colonies. White to deep yellow colonies that developed on the Mannitol salt agar plates were isolated and sub-cultured to obtain pure colonies. Red colonies with black centres that developed on the plates were isolated and sub-cultured to obtain pure colonies. Pure colonies from the different media were counted using standard plate count method. Confirmatory tests for *Escherichia coli, Staphylococcus* sp, and *Salmonella* sp. were carried out according to ISO 18593 (2004).

Antibiotic sensitivity test

The antibiotic sensitivity of the 3 bacterial isolates (*Escherichia coli, Staphylococcus* sp. and *Salmonella* sp.) to 14 antibiotics: Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Gentamycin (10 µg), Ampicillin (10 µg), Augmentin (30 µg), Ceftriaxone (30 µg), Streptomycin (10 µg), Ceftazidime (5 µg), Tetracycline (30 µg), Enrofloxacin (5 µg), Amoxicillin (10 µg), Penicillin (10 µg), Septrin (30 µg), Erythromycin (10 µg), was determined by the standard disk-diffusion technique in Mueller-Hinton agar (Clinical and Laboratory Standards Institute, 2013).

Determination of virulence genes

The genomic DNA of the bacterial isolates: *Escherichia coli* (11)*, Staphylococcus* sp. (9), *Salmonella* sp. (15), was extracted using ZYMO (ZR) bacterial genomic DNA extraction kit (Zymo Research, U.S.A.) following the manufacturer's instructions. The presence of three virulence genes *tsh*, *hlg*, and *invA* (which enhance virulence and pathogenicity) in *E. coli*, *Staphylococcus aureus* and *Salmonella* sp. isolates respectively were detected by polymerase chain reaction (PCR). Amplification of the genes was achieved by employing the specific primers corresponding to the virulence genes. PCR was performed in a total reaction volume of 10 µl containing 1.5 µl of template DNA (1 µg), 5.0 µl of 2×PCR master mix (Norgen Biotek Corporation, Canada) which contains Taq DNA polymerase, dNTPs, reaction buffer, $MgCl_2$, KCl and PCR enhancer/stabilizer; 1.0 µl of forward primer (2.5 µM), 1.0 µl of reverse primer (2.5 µM) and 1.5 µl of nuclease-free water. PCR reactions were carried out in a TC-412 Thermocycler (Keison, United Kingdom) employing the following amplification conditions: Initial denaturation step of 95 ^oC for 2 minutes, followed by 35 amplification cycles each consisting of denaturation at 94 ^oC for 1 min, annealing for 60 seconds and extension or elongation at 72 ^oC for 2 minutes. Reactions were terminated at final extension of 72 ^oC for 10 minutes. The amplified products were analysed by electrophoresis on a 1 % (w/v) agarose gel, stained with ethidium bromide in the presence of a 1 kb PCR sizer ladder (Norgen Biotek Corporation, Canada). Electrophoresis was performed at 80 V for 60 minutes. The sizes were then read against molecular marker of known size by looking at the banding patterns received after gel electrophoresis results, and to observe the virulence genes of the different bacterial isolates (Oloyede *et al*., 2016).

Statistical analysis

All data were analysed using the IBM Statistical Package for Social Science (SPSS) software. Data were expressed as mean ± Standard Deviation. Analysis of variance (ANOVA) was used to determine if the variation observed between variables is significant. The p-value > 0.05 was considered not statistically significant (Ogbeibu, 2015).

Results and discussion

In this study, the bacteria isolated were *Escherichia coli*, *Salmonella* sp and *Staphylococcus aureus* (Table 1). Results are similar to that reported by Kayode (2014), who observed that *Escherichia coli, Salmonella* sp.*, Proteus* sp.*, Klebseilla* sp.*, Pseudomonas* sp.*, Enterobacter* sp.*, Streptococcus* sp.*, Shigella* sp.*, Staphylococcus* sp.*, Bacillus* sp. and *Clostridium* sp. where isolated and identified in Kara and Odo-eran abattoirs in Ogun state (Nigeria). Also, Itah *et al.* (2005), isolated *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Staphylococcus epidermidis, Micrococcus roseus, Bacillus subtilis*. Species of *Streptococcus, Klebsiella, Pseudomonas* and *Salmonella* from Uyo abattoir (Nigeria).

Table 1.

Morphological and biochemical characteristics of bacterial isolates.

TEST	MacConkey Agar	Mannitol Salt Agar	XLD agar
Colony	Pinkish	Yellow/Milky	Reddish
Gram stain	-	+	-
Shape	Rod	Cocci	Rod
Arrangement	Single	Irregular	Single
Lactose	+	+	-
Indole	+	-	-
Oxidase	-	Nil	-
Citrate	-	Nil	Nil
Catalase	+	+	Nil
Coagulase	-	+	Nil
Mannitol	Nil	+	Nil
Hydrogen	-	-	+
Urease	-	Nil	-
Motility	Motile	Nil	Motile
Isolates	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella* sp.

Results revealed that the heterotrophic bacterial count from the processing halls ranged from 3.47 ± 0.19 x 10^3 to 9.28 ± 0.26 x 10^3 cfu/cm^2. It was observed that the *E. coli* count, which ranged from 2.50± 0.40 x 10^3 to 2.91± 0.22 x 10^3 cfu/cm^2, was higher than those of *Staphylococcus aureus* (0.55± 0.06 x 10^3 to 1.97± 0.70 x 10^3

cfu/cm^2) and *Salmonella* sp. (0.77 ± 0.08 x $10^3$to 2.52 ± 0.74 x 10^3cfu/cm^2) (Table 2). From the sales tables in the abattoir, similar results were obtained, as the *E. coli* count (0.74 ± 0.37 x 10^3 to 4.07 ± 0.81 x 10^3cfu/cm^2) was higher than the *Staphylococcus* (1.28 ± 0.38 x 10^3 to 2.11 ± 0.49 x 10^3cfu/cm^2)and *Salmonella* (1.24 ± 0.44 x 10^3 to 1.94 ± 0.63 x 10^3cfu/cm^2) count, while the heterotrophic bacterial count ranged from 6.29 ± 1.25 x 10^3 to 7.97 ± 0.03 x 10^3cfu/cm^2) (Table 3). This is not surprising as *E. coli* is an enteric organism and would have come from the intestinal tract and faecal matter of the slaughtered animals (Jay, 2005). This also implies that *E. coli* is the fastest in colonizing the environment. Studies have shown that pathogenic microbes especially *E. coli* shed by animals can persist in soil, water, manure, and feed, where it can spread to other uninfected animals (Hancock *et al.,* 1997) and to humans (Dos Santos *et al.,* 2007).

Table 2.

Bacterial load of processing halls at the abattoirs.

Abattoirs	Heterotrophic bacterial count x10^3 cfu/cm^2	*E. coli* count x10^3 cfu/cm^2	*S.aureus* count x10^3 cfu/cm^2	*Salmonella* count x10^3 cfu/cm^2
AB 1 (n = 2)	7.84 ± 2.08	2.90 ± 0.23	1.97 ± 0.70	2.52 ± 0.74
AB 2 (n = 2)	3.47 ± 0.19	2.91 ± 0.22	0.55 ± 0.06	0.77 ± 0.08
AB 3 (n = 2)	7.19± 0.94	2.66 ± 0.35	1.26 ± 0.06	1.51 ± 0.06
AB 4 (n = 2)	9.28 ± 0.26	2.50 ± 0.40	1.86 ± 0.51	1.71 ± 0.47
p-value	0.047	0.811	0.146	0.138

Key: AB= Abattoir, n = number of samples collected

Table 3.

Bacterial load of sales tables in the abattoirs.

Abattoir	Heterotrophic bacterial count (x10^3) cfu/cm^2	*E. coli* count (x10^3) cfu/cm^2	*Staphylococcus aureus* count (x10^3) cfu/cm^2	*Salmonella* count (x10^3) cfu/cm^2
AB 1 (n = 3)	7.43 ± 0.81	3.41 ± 0.46	2.11 ± 0.49	1.70 ± 0.30
AB 2 (n = 3)	6.29 ± 1.25	4.07 ± 0.81	1.28 ± 0.38	1.06 ± 0.15
AB 3 (n = 3)	8.21 ± 0.99	0.74 ± 0.37	1.71 ± 0.19	1.24 ± 0.44
AB 4 (n = 3)	7.97 ± 0.03	2.33± 0.32	1.83 ± 0.41	1.94 ± 0.63
p-value	0.475	0.010	0.522	0.466

Key: AB= Abattoir, n = number of samples collected

Escherichia. coli isolates were observed to be multi-drug resistant (resistant to at least three classes of antibiotics) (Table 4). They were resistant to ampicillin, tetracycline and ceftazidime. This characteristic resistance to ampicillin and tetracycline identified at a high rate, is similar to previous findings in *E. coli* isolates from diarrheic or diseased animals in China (Rehman *et al.,* 2017; Zhang *et* *al.,* 2017). *Salmonella* isolates in this study were resistant to a number of notable antibiotics. It was observed that they were resistant to ceftazidime (100%), ampicillin (83.3%) and chloramphenicol (79.2%). This implied multi-drug resistance, as the three antibiotics listed above were from different classes of antibiotics, with different mechanisms of action. This is similar to previous study, where Akbar and Anal, (2013) reported that all strains of *Salmonella* isolated from poultry in their study were resistant to three or more antibiotics. The resistance profilewere as follows: ampicillin (87%), chloramphenicol (63%), tetracycline (60%), trimethoprim (42%), sulphonamides (42%) and streptomycin (61%). The *Staphylococcus* isolates in this study were resistant to penicillin (100%), amoxicillin (85%) and augmentin (75%). The aforementioned antibiotics are all beta-lactams showing that these isolates are methicillin resistant. This result is consistent with the findings of Adesiji *et al.* (2011) who reported that *S. aureus* from retail meat products in Oshogbo, Nigeria were all resistant to amoxicillin. Their study also reported that *S. aureus* was susceptible to gentamycin, erythromycin and streptomycin which is in line with the findings of this present study.

In this study, 11 *E. coli* isolates were screened for the *tsh* (temperature sensitive haemagglutinin) virulence gene. The results showed that 7 (63.6%) out of the 11 were positive for the *tsh* gene (Figure 1). The *tsh* gene contributes to the development of lesions and deposition of fibrin in the avian air sacs (Kobayashi *et al.,* 2010). The *tsh* gene is mostly reported in APEC (avian pathogenic *E. coli*) strains (Saidenberg *et al.,* 2013) where it is believed to play a role in mechanisms of adherence to the respiratory tract of poultry (Dozois *et al.*, 2000).

Diseases caused by Staphylococci are the result of a synthesis of several virulence factors including the different hemolysins which are important for virulence of the *S. aureus* and other Staphylococci (da Silva *et al*., 2005). They're four types of hemolysins - alpha, beta, gamma and delta hemolysin produced by coagulase positive Staphylococci. Several studies indicated that hemolysins of *S.aureus* correlated well with infections in human and animals (Tackeuchi *et al.,* 2001; Larsen *et al.,* 2002). In this study, of the nine isolates screened for gamma haemolysin (*hlg*) virulence gene only 2 (22.22%) tested positive for *hlg* (Figure 2). In this study, *invA* (invasion protein) gene of *Salmonella* was investigated using *Salmonella* specific primers.

Of the 15 isolates screened for *invA* gene, 14 were positive and 1 was negative. The *invA*genes amplified by PCR was observed as 248bp amplicons (Figure 3). The *invA*, gene of *Salmonella* contains those sequences that are unique to this genus and has been proven to be a suitable PCR target with potential diagnostic applications (Jamshidi *et al.,* 2009). The *inv*A gene codes for protein in inner membrane of bacteria, which is necessary for invasion to epithelial cells (Shanmugasamy *et al.,* 2011). This gene is involved in the invasion of the cells of the intestinal epithelium and is present in pathogenic *Salmonella*. Therefore for salmonellosis to occur it is important that a gene responsible for invasion must be present. According to Zahraei *et al.* (2006), this gene is essential for full virulence in *Salmonella* and is thought to trigger the internalization required for invasion of deeper tissue.

Table 4.

Antibiotic sensitivity pattern of bacterial isolates

Antibiotics	*Escherichia coli* isolates		*Salmonella* isolates		*Staphylococcus* isolates	
	No. (%) of sensitive isolates	No (%) of resistant isolates	No (%) of sensitive isolates	No (%) of resistant isolates	No (%) of sensitive isolates	No (%) of resistant isolates
CHL	10(52.6%)	9(47.4%)	5(20.8%)	19(79.2%)	16(80%)	4 (20%)
CPR	12(63.2%)	7(38.8%)	7(29.2%)	17(70.8%)	14 (70%)	6 (30%)
GEN	15(78.9%)	4(21%)	13(54.2%)	11(45.8%)	20(100%)	0
AMP	0	19(100%)	4(16.7%)	20(83.3%)	-	-
AUG	10(52.6%)	9(47.4%)	11(45.8%)	13(54.2%)	5(25%)	15(75%)
CTR	5(26.3%)	14(73.7%)	22(91.7%)	2 (8.3 %)	-	-
STR	15(78.9%)	4(21 %)	14(58.3%)	10(41.7%)	12(60%)	8(40%)
CAZ	1(5.2%)	18(94.7%)	0	24(100 %)	-	-
TET	0	19(100%)	15(62.5%)	9(37.5%)	11(55%)	9(45%)
ENOA	19(100%)	0	13(54.2%)	11(45.8%)	-	-
MC	-	-	-	-	3(15%)	17(85%)
PEN	-	-	-	-	0	20(100%)
SXT	-	-	-	-	11(55%)	9(45%)
ERY	-	-	-	-	19(95%)	1(5%)

Key: CHL= Chloramphenicol, CPR = Ciprofloxacin, GEN = Gentamycin, AMP = Ampicillin, AUG = Augmentin, CTR = Ceftriaxone, STR = Streptomycin, CAZ = Ceftazidime, TET = Tetracycline, ENO = Enrofloxacin, AMC = Amoxicillin, PEN = Penicillin, SXT = Septrin, ERY = Erythromycin.

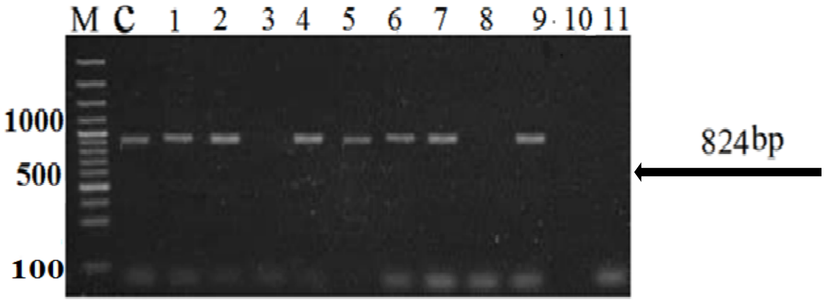

Figure 1. Gel electrophoresis of *E. coli tsh* virulence gene PCR products. Lane M: 100 bp marker, Lane C: positive control, Lanes 1, 2, 4, 5, 6, 7 and 9 indicate positive bands for *E.coli tsh* gene, Lane 3, 8, 10 and 11 are negative bands for *E. coli tsh* gene.

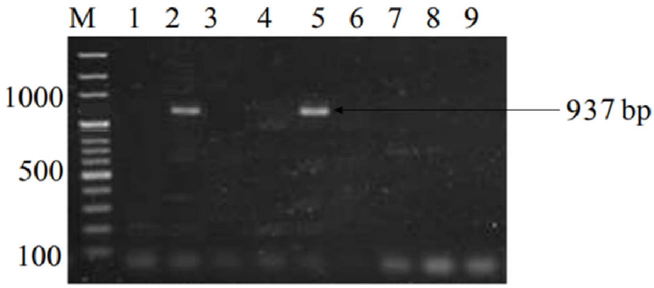

Figure 2. Gel electrophoresis of *Staphylococcus* spp. *hlg* virulence gene PCR products. Lane M: 100 bp marker, Lanes 2 and 5: positive bands for *hlg,* Lane 1, 3, 4, 6, 7, 8, and 9 are negative for *hlg*.

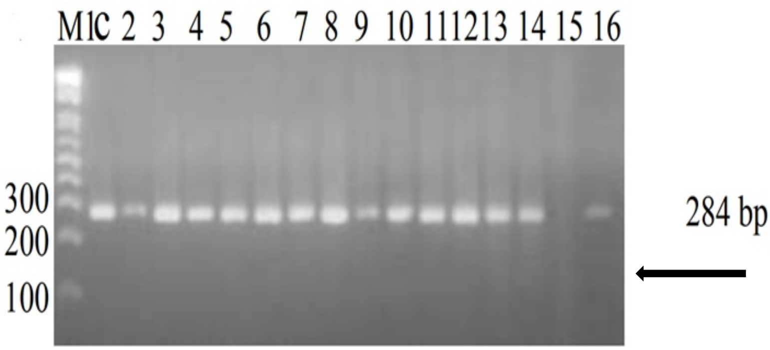

Figure 3. Gel electrophoresis of *Salmonella inv*A virulence gene PCR products. Lane M: 100 bp marker, Lane 1C: positive control, Lanes 2-14 and 16: *invA* gene band. Lane 15 is negative for *invA* virulence gene.

Conclusions

Pathogenic bacteria detected in abattoirs could pose great risk to public health, especially when they posess antibiotic resistance genes and virulence factors. It is recommended, therefore that beef should be properly washed and cooked adequately before consumption. Public enlightenment and proper monitoring of meat and meat products, as well as implementation and surveillance of hygiene measures through the processing and selling by food regulatory bodies is also advised.

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