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Persistence and Biofilm Assessment of *Campylobacter Jujeni* in Poultry Abattoir

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ABSTRACT

Persistence of *Campylobacter* sp and its biofilm forming ability was assessed in two poultry abattoirs at two weeks intervals. Average prevalence (63.75%) of *Campylobacter* spp. was observed on assessing a total of 160 samples collected from the surfaces of packaging table (80%), dressing table (75%), floor source (70%) and washing table (30%). Biofilm assessment formed by *Campylobacter jejuni* within 5-days at 37^0C were in decreasing order of washing table> packaging table> dressing table > floor. An average rate (19.6%) of isolates to develop biofilm observed in both sites was considered relatively low. Absorbance value (Optical Density-OD_{590nm}) of formed biofilms ranged from 0.483 – 0.952. Wastewater from the facilities showed higher TDS (643 – 820 mgl^{-1}), TSS (1200 – 1775 mgl^{-1}), COD (152 – 141 mgl^{-1}) and BOD (30.3 – 32.5mgl^{-1}) than the WHO standards of 500 mgl^{-1}, 100 mgl^{-1}, 10 mgl^{-1} and 6 mgl^{-1} respectively. This is a clear indication of heavy microbial presence in the wastewater. Total bacterial count (TBC) was slightly higher in site A (4.4×10^5 CFU/ml) than site B (3.5×10^5 CFU/ml). Efficiency index ratio (≈/>1) observed in all tested drugs suggests their effectiveness in campylobacteriosis management. Decreasing drug sensitivity pedigree was observed with streptomycin> erythromycin & gentamincin > tetracycline & neomycin > penicillin> riphapicin & ampicillin > norflaxicin & cephalexin. These results of frequency and biofilm forming tendencies of *Campylobacter* spp. observed in this study can be of value in checkmating campybacteriosis outbreak from poultry abattoir facility.

Keywords: *Campylobacter jejuni,* biofilm, poultry, abattoir.

Introduction

Public health concern has been drawn to the sporadic cases of *Campylobacteriosis*. Universally, *Campylobacter* spp have recorded high prevalence in poultry and its products (Mackiw and Szponar 2007; Shane 2000). C. *jejuni*, has the highest prevalence in outbreaks of *Campylobacteriosis* and thus serves as prototype for *Campylobacters* (Tauxe, 1992). Mboto *et al.*, (2012) report state that *C. jejuni* is among the emerging food-related pathogens. Ability of this pathogen (*C. jejuni*) to grow optimally at 37^0 to 42^0C (Nachamkin, 2003), accounts for its high prevalence on the skin surface of poultry with approximate temperature of 41 – 42^0C. Processes at the abattoirs are mainly aimed at reducing the bioload of pathogens present on the carcass. However, Stern *et al.,* (1995) reported that processing of poultry in abattoirs have increased the bioload of *C. jejuni* in 10 – 1000 folds. Atuanya *et al.,* (2012) and Hinton *et al.* (2000) are of the opinion that slaughtering of animals in most abattoirs of developing countries are carried-out in unsuitable buildings by untrained slaughter-men and butchers that are unaware of

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sanitary principles. Assessment on necks and breast of birds (Samelis and Metaxopoulos, 1999), the handlers and poultry environment (Clouser, 1995) were noted as sources of abattoir contamination.

Formation of biofilm within the abattoir environment has enhanced bacterial persistence and bioload. This interactive matrix of aggregate microbes encased in an exopolymer compounds limits the destruction of these microbes by antibiotics, desiccators, sanitizers, mechanical damage and protozoan predators (Mosterller and Bishop, 1993), Ultra violet (UV) rays and heat treatments (Hall-stoodley *et al.*, 2004). *Campylobacter jejuni* was found to form biofilm in both monoculture and mixed culture media (Teh *et al.*, 2010; Joshua *et al.*, 2006) and this ability is enhanced in aerobic condition (Reuter *et al.*, 2010). Though, Wirtanen and Salo (2005) and Hall-stoodley *et al.*, (2004) argues that every microorganisms have varying ability to form biofilm under favourable conditions (moist surface, nutrient and physiological environments). Selective surfaces prone to form biofilm that were assessed by this study include dressing tables, packaging tables, washing tables and the facility floor. Other surfaces include joints, valves, cutting utensils and several dead ends (Ellebroek, 1997). Persistence of *C. jejuni* would be greatly enhanced by its ability to form biofilm on surfaces of food and processing environments. This poses a difficult challenge for campylobacteriosis management. Thus, this paper assessed the prevalence of *Campylobacter* spp. in two indigenous poultry abattoirs and ability of the isolates to develop biofilm within 5 days.

Material and Methods

Sample collection

A total of 160 samples were collected from two indigenous poultry abattoirs in Port Harcourt metropolis, Rivers State, located within the longitude $7^0$9'E and latitude $4^0$4'N. Two (2) samples (before and after poultry processing) were collected each from the surfaces of dressing table, packaging table, washing tubs and the facility floor. Samplings were replicated ten (10) times on both sites at two weeks interval. These surfaces were swabbed diagonally in opposite direction with commercial swab sticks and kept in ice bath ($<4^0$C) while on transits to the laboratory for analysis.

Isolation and identification

Each Swab sample was pre-enriched in 10 ml of Hunt Enrichment broth (Becton and Dickinson, Oxoid) for 24 h at 37^0C. Filter membranes with pore size of 0.6 μm and 50 mm diameter (Schleicher and Schuell ME 26) were placed on Tryptose Blood Agar plates (CM 233, Oxoid) containing 10% unlysed horse blood. Few drops (2 – 3) of pre-enriched samples were placed onto filters and left for 30 minutes. The filters were then removed and the plates were incubated at 37^0C for 48 h in a microaerophilic atmosphere (5% O_2, 10% CO_2, and 85% N_2) using Oxoid gas pack (BR 38) (Le Roux and Lastovica, 1990). *C. jejuni* was isolated in a selective medium using Skirrow's medium at 42^0C to inhibit other members of *Campylobacters* (which normally grow at 36 – 37^0C). Isolates were then gram stained for the Gram negative gull wing shaped rods (Nachamkin, 2003).

Determination of total viable bacterial count (TVBC)

Total bacterial counts of wastewater samples from the two sites were determined by the method described by Adesemoye *et al.* (2006). The waste samples were serially diluted to 10^{-4} and 0.1 ml was aseptically inoculated on a sterile nutrient agar plates and incubated at 30^OC for 24 h. Plates with distinct colonies were counted, and total bacteria was estimated and recorded as colony forming units per ml.

Biofilm assessment

Five glass slides (6 x 2 cm^3) were aspetically placed inside a jar containing 500 ml of Tryptic Soy Broth TSB (CM 129, Oxoid). Exactly 5 ml of 18 – 24 h old broth culture of the isolates were inoculated into TSB and incubated for 5 days at 37^0C. After this period, the glass slides were treated with phosphate buffered saline to remove unattached cells, followed by air drying and later heat fixed to avoid mechanical damage during staining. Crystal