ARCOBACTER BUTZLIERI STRAINS FROM POULTRY ABATTOIR EFFLUENT IN NIGERIA

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ABSTRACT

Objective: To investigate the prevalence, species distribution and genetic diversity of zoonotic Arcobacter species.

Design: Prospective study.

Setting: Drainage system of a cosmopolitan chicken abattoir in Lagos, Nigeria.

Methods: One hundred and fifty drainage water samples were enriched in a minimal antibiotics-containing medium at room temperature and bacteria then isolated by use of a membrane filtration method.

Results: Twenty six (14%) of samples were positive for Arcobacter spp. Of these, 20 were examined by a comprehensive probabilistic identification scheme for Epsilobacteria and all strains identified as A. butzleri. AFLP analysis of these strains revealed considerable genetic diversity among the strains, with 12 genotypes defined at the 90% similarity level.

Conclusion: The prevalence of A. butzleri in Nigerian poultry abattoir effluent indicates this species may constitute a public health problem in this country. AFLP profiling could be a useful tool for molecular epidemiological and population genetic studies of this organism. This is the first known report of A. butzleri in Nigeria, and first application of AFLP analysis for genotyping the species.

INTRODUCTION

Arcobacter species are metabolically inactive, curved or spiral-shaped bacteria initially classified as Campylobacter species (1), although their ability to grow in air at low temperature (30°C) after primary isolation in microaerobic conditions was a marked difference. Organisms with this phenotype were later designated into a separate genus by Vandamme et al. (2) based on evidence from immunotyping, SDS-PAGE protein profiling, and DNA-DNA- and DNA-rRNA hybridization experiments. Four species are presently recognised: Arcobacter cryaerophilus [formerly Campylobacter cryaerophilus; (3)], A. nitrofigilis [formerly C. nitrofigilis; (4)], A. butzleri [formerly C. butzleri; (5)], and A. skirrowii (6). Of these species, only A. nitrofigilis has not been associated with human and/or animal disease.

The socio-economic importance of Arcobacter spp. initially stemmed from their frequent association with diseases of livestock. These include mastitis in milk producing cows, septic abortion in cattle and pigs, diarrhoea, and infertility problems in cattle (3,7-9). However, since re-classification(6), Arcobacter spp. have emerged from obscurity to be recognised as food borne pathogens of public health importance (10). Outbreaks and sporadic case reports of human Arcobacter infections are mainly those of gastroenteritis (5,11-13).

Arcobacter spp have been isolated from a wide range of food and water sources. They have been detected in poultry and animal carcasses (14,15), sewage, wells, rivers and drinking water (16-18). It has been suggested that Arcobacter gastroenteritis may be more prevalent in developing countries because of the poor level of hygiene and inadequate supply of good drinking water (19).

In this study, we investigated the presence of Arcobacter spp in drainage water samples from the washings of chicken carcasses in a Nigerian abattoir, and characterized strains by phenotypic and genotypic methods to determine their taxonomic and genomic diversity.

MATERIALS AND METHODS

Samples and location studied: Untreated wastewater effluents of a chicken abattoir in Lagos metropolis (Mushin) were used. Different birds were slaughtered in this abattoir and wastewaters from the washings of their carcasses discharged directly into a shallow open drainage that are linked to a major drainage system.

Collection and enrichment of samples: One hundred and fifty 5ml samples of wastewater effluent were collected at random over a period of four months (May-August 2000) and
transported within 30 min of collection to the laboratory. About 0.5 ml of slightly turbid supernatant of the test samples were added to 4.5 ml sterile brain-heart infusion broth (Oxoid Ltd., Basingstoke, UK) containing 0.5% yeast extract, Campylobacter selective antibiotic supplement (SR 155, Oxoid), giving final concentrations of 0.032 mg/ml cephaloridine and 0.01 mg/ml amphotericin, and 0.01 mg/ml pharmaceutical vancomycin hydrochloride (American Pharmaceutical Partners Inc., Los Angeles, USA) (BHI-CVA broth). The antibiotic formulation concurred with commercially produced cephaloridine-vancomycin-amphoterin (CVA) media that is used for isolation of Arcobacter spp. (20). Cultures were incubated for 18-20 hours at room temperature (28-29°C) with the caps of the containers slightly loosened to select for aerobic growth at low temperature (21).

Isolation of Arcobacter strains from enrichment culture: 1 ml aliquots of enrichment culture were diluted in sufficient sterile nutrient broth so as to produce a faintly turbid suspension and then inoculated by both direct and membrane filter techniques onto BHI-CVA agar media containing 5-7% v/v sheep blood. For membrane filter isolation, sterile 0.45µm pore sized cellulose acetate membrane filters (Advantec Ltd., Tokyo, Japan) were placed onto the media and 5-7 drops of diluate added onto the filter surface. Membrane filters were then removed aseptically after incubation for 30 min at room temperature. All inoculated plates were incubated in a candle extinction jar (22) for 24-48 hours at 37°C. Plates with poor growth but which did not show presence of Proteus or confluent growths of contaminants were usually incubated for an additional 24 hours. Bacterial colonies on primary isolation plates that resembled those of Campylobacter species were subcultured for further testing.

Identification of isolates: Isolates were presumptively classified as Arcobacter spp. by determination of, (i) cell morphology through examining gram-stained films (using strong carbol-fuchsin as counter stain) under light microscopy, (ii) oxidase and (iii) catalase activity, (iv) ability to ferment glucose and lactose and (v) growth in atmospheric air at room temperature (28-29°C) by use of previously described methods, (14,23). Motility, and urease and indole production were determined by use of motility-indole urea medium (Bio-Life, Ljusne, Sweden) according to the manufacturers instructions. Gram-negative curved or spiral rod-shaped, motile, aerotolerant, asaccharolytic bacteria that grew on MacConkey agar and produced oxidase and catalase but not urease or indole were considered potential Arcobacter spp. Species identity of 20 of the isolates was determined by extensive (>60 traits) phenotypic characterisation and probabilistic comparison of data with similar results for 37 Campylobacter, Arcobacter, Helicobacter and related taxa, as described previously (24).

AFLP profiling: The genetic diversity of 20 strains was examined by Amplified Fragment Length Polymorphism (AFLP) analysis by use of the methods described previously for Campylobacter spp. (25). Digitized patterns were analysed by use of the program BioNumerics 2.5 (Applied Maths, Kortrijk, Belgium). Bands were defined by use of automated search parameters detecting fragments with a minimal area of 1.5% of total pattern area, an intensity of 9.0% greater than the background fluorescence and a shoulder sensitivity of 5.0 to improve detection or hands with near-identical molecular sizes. Similarities between strain profiles were calculated using the Dice coefficient and strain relationships inferred by Unweighted Pair-Group Mathematical Average (UPGMA) clustering. Strains 8, 16, 18, 20, 41 and 45 were examined on two different occasions to determine reproducibility of the AFLP analysis.

RESULTS

An enrichment method in conjunction with membrane filtration was found suitable for isolation of Arcobacter species from wastewater effluent of chicken abattoir. Twenty six (14%) of the samples examined were positive for Arcobacter strains based on presumptive identification by the salient phenotypic criteria described above. Twenty strains were chosen at random for extensive phenotypic characterisation, of which, 19 had identification (ID) scores (Willcox probability percentage values) to A. butzleri exceeding 98% while one isolate (Lagos 26) attained an ID score of 83% to this species.

Figure 1

AFLP band patterns of 20 Nigerian Arcobacter butzleri strains. The scale bar denotes percentage similarity between patterns based on the Dice coefficient and UPGMA clustering.
low. By contrast, use of an enrichment method in conjunction with membrane filtration resulted in the recovery of 26 strains (representing 14% of the samples examined) that were presumptively classified as Arcobacter spp. by salient phenotypic criteria as described above. Our results further illustrate the suitability of membrane filtration for isolation of Arcobacter spp, and other campylobacteria with fastidious growth requirements and/or marked susceptibility to antibiotics used in selective media (17,18,20,27,28).

Accurate identification of Arcobacters and related Epsilonobacteria (including Campylobacter, Helicobacter and other organisms: (29) is well known to be problematic (24). This factor may have contributed to the limited information concerning the natural habitat, incidence and modes of transmission of Arcobacter species to man and animals (14). In this study, each of the 20 strains chosen at random for extensive phenotypic characterisation were confidently identified as A. butzleri by the scheme of On et al. (30), with identification (ID) scores (Wilcoxon probability percentage values) to this species exceeding 98% for 19/20 strains. One isolate (Lagos 26) attained an ID score of 83%, to A. butzleri, a result due to atypical (all negative) results in triphenyl-tetrazolium chloride, potassium permanganate and nalidixic acid tests. Nonetheless the data illustrate the efficacy of this approach for identifying Epsilonobacteria, as demonstrated in previous studies (14,31).

The predominance of A. butzleri in the poultry abattoir effluent in this study resembles that seen in studies of poultry and poultry products, where this species is significantly more common than other Arcobacter spp. (14,15,21,32). Similarly, A. butzleri is the sole or predominant Arcobacter species recovered from drinking and river water (17,33,34), indicating effective adaptation to survival and dissemination in an aqueous environment; observations with epidemiological implications to both animals and humans. Although A. butzleri appears sensitive to disinfectants used to treat sewage (35), transmission via contaminated water is clearly a risk where exposure to untreated water occurs. Such conditions are likely in animal housing facilities and in countries with poorly developed sanitation conditions Arcobacters are psychrophilic they tend to grow best at temperatures lower than 30°C and can even grow at 15°C. Although most strains grow at 37°C, relatively few (25%) grow at 42°C (30). Thus their observation in a tropical country such as Nigeria is actually a rather interesting extension of our knowledge of their ecology.

These data illustrate the public health risk potential of A. butzleri and it is noteworthy that this species was recently isolated from 24.6% of human diarrhoea cases using appropriate isolation methods (36).

Few studies have examined the genetic diversity of A. butzleri. Ribotyping of 64 unrelated strains by their epidemiology revealed 50 different patterns, of which strains from the USA examined appeared to be more conserved than those studied From Australia, Thailand and Northern Ireland (19). PCR fingerprinting using repetitive sequences allowed discrimination of outbreak strains from 10 unrelated isolates, although considerable similarity among the patterns was seen (37). The efficacy and sensitivity of AFLP profiling for genotyping is well established (38) and we believe our study is the first to describe the application of AFLP fingerprinting to A. butzleri. This method was first developed for Campylobacter spp. (25) and its efficacy for examining A. butzleri suggests it may be useful for characterizing other members of the Campylobacteraceae including Sulfospirillum and Bacteroides ureolyticus. Reproducibility of the AFLP assay was determined as 89.95% (± SD 1.48) and its high discriminatory potential is confirmed by recognition of 12 genotypes (among 20 A. butzleri strains) at the 90% similarity level (Figure 1). Thus, AFLP is a promising method for epidemiological investigations of this species.

Many common fragments were observed in the AFLP profiles (Figure 1) an observation consistent with the strains belonging to a well-defined species and possibly indicating a clonal relationship among the strains. The latter is to some extent corroborated by previous studies, (19,37), but more data are required to better establish the level of genetic diversity and implications for population structure of A. butzleri. Further studies are underway.

It is possible that the prevalence of Arcobacter species reported here is an underestimate. The candle jar method used here is inexpensive and often used in developing countries but does not provide an optimal microaerobic environment (39). Furthermore, cultivation of certain Arcobacter spp. (notably A. skirrowii) is more difficult than others (14,32), Nevertheless our report is, to our knowledge, the first to confirm these bacterial pathogens in Nigeria and it thus complements some two decades of studies on Campylobacter in the country (40). The accumulation of data indicating the zoonotic and pathogenic potential of these organisms supports the need for further studies of their prevalence, distribution and significance in both developing and developed countries. Additional studies on the ecological diversity; and the public health importance of the organism in Nigeria are in progress.

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REFERENCES


Dear Sir

Several investigators have recently addressed the issue of unexpected airway management problems caused by preoperatively unrecognized presence of ethnic/fashion-related decorative hairstyles(1-3), mandibular cloth bands(4) and facial body art/body piercing(5,6) in patients presenting for emergency surgeries.

Elaborate hairpieces used to be worn only on formal occasions(2,3). However, there is a recent trend wherein African-Americans and African-European women are increasingly wearing elaborate hairpieces even in the absence of a formal indication. These hairpieces (Figure 1) are firmly tied to the native hair and their quick removal in an emergency is difficult. Additionally, hospital head covers may mask the problem (Figure 2).

Chikungwa reported airway management problems resulting from elaborate pom-pom hair style in an African woman in Harare, Zimbabwe(2). Ashley and Marshall encountered similar hairstyle-related airway management problems in African British women in London, England(1). Kuczkowski and Benuomof described problems with airway management and unexpected difficult intubation resulting from decorative hairstyle in the parturient in San Diego, USA(3). The authors concluded that their preoperative airway evaluation now includes examination of the hair formation(3).

Sinha et al.(6) described similar problems with unexpected difficult intubation resulting from a cloth band around the head worn by their patient for religious purposes. Body piercing is a form of body art. In the past, body piercing (including oral and nasal tissue piercing) was seen mainly in native tribes in Africa(5, 6). However, in recent years body piercing has gained increasing popularity worldwide and has become a firm fixture in western fashion trends.

Kuczkowski et al(5) described a case of an obstetric patient who presented for emergency postpartum surgery with fixated tongue jewellery in-situ (Figure 3), which resulted in trauma to the tongue and difficult airway management. The difficult airway management consisted of tongue bleeding at the time of laryngoscopy and tongue oedema at the time of extubation. The authors considered these two events to be near misses of “cannot intubate” and “cannot ventilate” situations, respectively, in a patient with otherwise no preoperative predictors for difficult airway.

Kuczkowski et al also reported another case of a parturient who presented for an emergency Caesarean section with nasal jewellery in-situ (Figure 4), which was unnoticed preoperatively and became externally loose intraoperatively(6). This finding necessitated fiberoptic examination of the nasopharyngeal and oropharyngeal cavities and radiological imaging studies under anaesthesia in order to rule out aerodigestive tract aspiration of retained and missing piece(s) of the jewellery prior to extubation.

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**Figure 1**
Model with a hairpiece very similar to the one actually worn by the patient described in report # 3

**Figure 2**
Elaborate hairpiece masked by the hospital-issued head cover

**Figure 3**
Model with tongue jewellery very similar to the one actually worn by the patient described in report # 5
Since the popularity of body piercing is increasing in many societies, it is reasonable to expect that the incidence of oral and nasal jewellery interference with airway management will increase (5, 6). Whether patients should be routinely asked to remove oral jewellery prior to a delivery, an operative delivery, administration of labour analgesia, surgical anaesthesia of any kind is debatable. Kuczkowski et al concluded that on the basis of this experience, their pre-anaesthetic evaluation now includes specific questioning for the presence of oral or nasal jewellery (3,4). At the University of California, San Diego all labouring patients with oral or nasal jewellery are advised to remove the hardware while in labour and particularly prior to administration of labour analgesia or anaesthesia, for safety considerations.

In obstetric patients, airway engorgement may further increase the severity of trauma-related bleeding or swelling caused by the presence of oral or nasal jewellery. Patient’s reluctance to remove the jewellery because it would be difficult to re-insert is common. Moreover, emergency obstetric cases may preclude having sufficient time to safely remove the hardware.

In conclusion, due to constantly increasing ethnic and cultural diversity in our society, it seems logical to re-emphasize that ethnic and fashion trends in hairstyle, dress, and body art should be identified and taken under consideration when performing preoperative evaluation for anaesthesia.

Yours sincerely

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