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ARTICLE

Isolation of *Tatumella ptyseos* from Beef in Ibadan, Nigeria

*[1]AGBAJE, M., [2]DIPEOLU, M. A., [1]OYEKUNLE, M. A., [3]GRACE, D. and [1]OJO, O.E.

[1]Department of Veterinary Microbiology and Parasitology, [2]Department of Veterinary Public Health and Reproduction, College of Veterinary Medicine, University of Agriculture, Abeokuta, Nigeria. [3]International Livestock Research Institute, Nairobi, Kenya.
*Correspondence: mikeagbaje@yahoo.com Tel: +234 8035 120645.

SUMMARY

Two hundred beef samples randomly collected from Bodija market in Ibadan, Nigeria were investigated bacteriologically. Four isolates resembling members of the *Enterobacteriaceae* family by phenotypic characterisation were further examined on MacConkey agar (MCA), Columbia agar with 5% ovine blood (CA) and biochemically. All the four isolates were identified as *Tatumella ptyseos* using the classical method and an online Advanced Bacterial Identification Software (ABIS). The susceptibility of the four isolates to antimicrobial agents was determined using the disk diffusion method. All the four isolates grew under aerobic conditions with no haemolysis on the CA supplemented with 5% ovine blood and as non-lactose fermentersion on MCA. Colonies were 0.5 - 1mm in diameter on CA with smooth and entire edge after 24hr incubation. The isolates were Gram negative bacilli, motile at 25°C but not at 37°C, oxidase negative, catalase positive, Simmon citrate positive at 25°C, but not at 37°C and phenylalanine deaminase positive. The isolates were sensitive to gentamicin, oxytetracycline, ciprofloxacin, norfloxacin, penicillin, enrofloxacin and amoxycillin but resistant to sulphamethoxazole. The homogeneity of all four isolates with regards to cultural and biochemically characteristics, cell morphology and antimicrobial susceptibility probably suggests a single species and strain. There is no information on previous isolation of this organism from food including meat or clinical samples in Nigeria. The identification of *T. ptyseos* in fresh beef in Nigeria as well as other food sources around the world in addition to its implication in human infections should be of interests to scientists especially, given the pathogenic potential of this organism.

KEY WORDS: Beef, *Tatumella ptyseos*, *Enterobacteriaceae*, Ibadan

INTRODUCTION

Tatumella ptyseos which was first characterized by Hollis (1981) derived its name from Harvey Tatum (a CDC microbiologist) and *Ptyseos*, epithet of sputum (Hollis *et al*., 1981). It is a relatively unknown new member of the *Enterobacteriaceae* family which was previously thought to belong to an unclassified bacterial group known as EF-9 (Hollis *et al*., 1981; Farmer *et al*., 1985). Following extensive studies, the name *Tatumella ptyseos* was proposed and subsequently adopted (Hollis *et al*., 1981).

Tatumella ptyseos is oxidase negative and has biochemical, cultural and morphological characteristics similar to those of the *Enterobacteriaceae*. Characteristic reactions of *Tatumella ptyseos* include positive reaction with Simmon's citrate and weak motility at 25°C but negative and non-motile at 37°C. While other motile members of the *Enterobacteriaceae* exhibit peritrichous flagellation, *T. ptyseos* only has one flagellum per cell which can be polar, sub-polar or lateral. It has also been observed that *T. ptyseos* does not survive long in laboratory media and generally has large inhibition zones with penicillin when compared to other members of the *Enterobacteriaceae* family (Hollis *et al*., 1981; Farmer *et al*., 1985). Although *T. ptyseos* is rarely reported as a cause of human infections, it has been associated with infection in immuno-compromised patients, mainly suffering from tuberculosis and diabetes (Tan *et al*., 1989). Neonates and the elderly are vulnerable to *T. ptyseos* (Bouvet and Grimont, 1987; Eisenstein, 1995). *Tatumella ptyseos* has also been implicated in opportunistic infections of the respiratory tract (Bilgehan, 1996).

This study reports the presence of *T. ptyseos* as contaminant in beef processed and sold for human consumption at the Bodija abattoir and a meat market in Ibadan, Oyo State, Nigeria. It also highlights the possible contamination of meat by human handling as well as the health implications of consuming such contaminated meat.

MATERIALS AND METHODS

This investigation was carried out at Bodija abattoir and a meat market located in Ibadan, the capital of Oyo State, south-western Nigeria. Ibadan is the largest city in West Africa and the second largest in Africa, with land size covering an area of 240 km^2 and human population of 1,222, 570 by 1991 census (Ayeni, 1994).

Sample collection and preparation

Meat samples were collected from meat processors and vendors in ten selected butchers association in the study location between October and December, 2009. A total of 200 raw pieces of meat samples were randomly collected (ten from each selected butchers' association) following adequate identification of meat processors and/or vendors to avoid sampling processors and/or vendors more than once. Sampling was done once in a week and mostly in the morning (between 9:00a.m and 11:00a.m) when meats were just freshly processed. Approximately 150g of raw beef samples were aseptically collected by asking processors to cut and place the pieces in sterile polythene bags. Samples were transported to the laboratory on ice (temperature of 0-4°C). Ten grammes (10 g) of each meat sample was weighed and homogenized in 90 ml of sterile distilled de-ionized water using a sterile warring blender. Ten fold dilutions of the homogenates were made using sterile pipettes as described by Fawole and Oso (2001).

Bacteriological analysis and identification

From the 10-fold dilutions of the homogenates; 0.1ml of 10^{-6} dilution of the homogenate supernatant for each sample was inoculated on plates of Columbia agar with 5% ovine blood (CA) (Oxoid®, Basingstoke, UK) and MacConkey agar (MCA) (Oxoid®, UK) in duplicates . The plates were then incubated at 37°C for 24-48h. After incubation, discrete colonies on agar plates were identified and purified by aseptically sub-culturing onto fresh sterile corresponding media to obtain pure secondary cultures of the isolates. The pure isolates were preserved in nutrient agar (Oxoid®, UK) slant and kept in the refrigerator at 4°C.

Colonies were carefully examined macroscopically for cultural characteristics such as shape, colour, size and consistency. Bacterial isolates were characterized based on microscopic morphology, colonial appearance, motility, Gram staining reactions and biochemical tests including oxidase, catalase, Triple Sugar Iron Agar (TSIA). Motility test was performed using the hanging drop method (Barrow and Feltham 2003). Catalase and Oxidase tests were performed as described by Shivaprasad (2003). Carbohydrate fermentation test was carried out as described by Cheesbrough (2000). Further biochemical tests were carried out using Microbact™ GNB, 24E (Oxoid, Basingstoke, UK) according to manufacturer's instructions. Isolates were identified on the basis of biochemical reactions in accordance with centers for disease control and prevention (CDC) Scheme for Identification of Gram Negative Bacilli (CDC, 1983) and also with the aid of on-line Advanced Bacterial Identification Software (ABIS).

Isolates from different samples were tested for antimicrobial susceptibility by the disk diffusion method on Mueller-Hinton agar, according to the Clinical and Laboratory Standards Institute (CLSI, formely NCCLS) (CLSI, 2003). The antimicrobials agents were: amoxicillin/-clavulanate 10 μg, cefotaxime, ciprofloxacin 5 μg, enrofloxacin 5 μg, gentamicin 10 μg, kanamycin 30 μg, norfloxacin 10 μg, penicillin 10 I.U., oxytetracycline 30 μg and trimethoprim/ sulphamethoxazole 25 μg.

RESULTS

Four isolates from four different samples out of the 200 samples analysed were identified as *T. ptyseos*. The organisms were Gram negative rods and produced non-lactose fermenting colonies on MacConkey agar as well as non-haemolytic colonies on Columbia agar with 5% ovine blood under aerobic condition. After 24h incubation on Columbia agar with 5% ovine blood, colonies were 0.5-1mm in diameter, smooth with entire edge. The organisms were motile and utilised citrate at 25°C but not at 37°C and produced other biochemical reactions which are characteristic of *T. ptyseos* (Table I). The isolates were all sensitive to gentamicin (10μg), oxytetracycline (30μg), ciprofloxacin (5μg), norfloxacin (10μg), penicillin (10 I.U) and amoxicillin/clavulanate (10μg). But they were all equally resistant to trimethoprim/-sulphamethoxazole (25μg).

Table I: Biochemical reactions of isolates

S/N	Test	Result/Reaction
1	Oxidase	(-)
2	Catalase	(+)
3	Triple Sugar Iron Agar	Yellow (Acid) slant/ Yellow (Acid) butt. No gas; No H_2S
4	Nitrate reduction	(-)
5	Simmons Citrate's Agar at 25°C	(+)
	37°C	(-)
6	Urease	(-)
7	Phenylalanine deaminase	(+)
8	Indole	(-)
9	Methyl Red	(-)
10	V-P	(-)
11	Gelatine hydrolysis	(-)
12	Esculin hydrolysis	(-)
13	Ornithine decarboxylase	(-)
14	Lysine decarboxylase	(-)
15	Arginine dehydrolase	(-)
16	Acid from Glucose	(+)
	Mannitol	(-)
	Xylose	(+)
	Sucrose	(+)
	Lactose	(-)
	Arabinose	(+)
	Adonitol	(-)
	Raffinose	(-)
	Malonate	(-)
	Inositol	(-)
	Sorbitol	(-)
	Rhamnose	(-)
17	Motility at 25°C	(+)
	37°C	(-)

Key: (+) = positive (-) = Negative

DISCUSSION

Although clinical information about *T. ptyseos* as a human pathogen is limited, this organism has been implicated in tracheotracheo-bronchial/-pulmonary infections such as pneumonitis, asthmatic bronchitis, pharyngitis, Wegener granulomatosis, pneumonia, chronic lung disease, and pulmonary edema (Hollis *et al.*, 1981; Farmer *et al.*, 1985; Stone *et al.*, 2007); pulmonary tuberculosis (Berka and Uzun, 2001) and gastrointestinal infection (Janda and Abbot, 2006). In addition this pathogen has been associated with sepsis in Malaysia (Tan *et al.*, 1989) and Brazil (Paulo *et al.*, 2008).

Tatumalla ptyseos is mostly isolated from human sputum (Farmer *et al.*, 1985) and blood from clinical cases (Tan *et al.*, 1989; Janda and Abbot, 2006); however, it has been isolated from other sources such as soil in Brazil (Silva *et al.*, 2007), vegetation in Japan (Hiroshi et al., 2006), edible macroalga (*Palmaria palmata*) in Ireland (Moore, 2002), water supplies in South Africa (Nevondo and Cloete, 1999) and poultry carcasses in Argentina (Jimenez *et al.*, 2003). Although there is currently very rare substantive evidence to suggest an association between this pathogen and cattle and or its by-product, the isolation of this pathogen in fresh beef in Nigeria largely incriminates the hygiene and processing procedure from a food safety stand point, as contamination could have resulted from infected human handling of meat and/or the environment. Also, reports of the isolation of *T. ptyseos* from various food sources around the world may well suggests the need for more surveillance and further studies especially as it regards public health importance.

In this study, all *T. ptyseos* isolates were sensitive to all the tested antibiotics except trimethopprim/sulphamethoxazole. Resistance of *T. ptyseos* to trimethoprim sulphamethoxazole has not been previously reported. However, resistance to ampicillin and imipenem has been observed in *T. ptyseos* (Rodríguez and Navarrete 2001; Stone *et al.*, 2007). In the study area, sulphamethoxazole is commonly used as first-line antibiotic in the treatment of the respiratory infection. Hence, human infection with sulphamethoxazole-resistant *T. ptyseos* strains may be refractory to treatment with this antibiotic.

The identical nature of the four isolates with regards to their cultural and biochemical characteristics, cell morphology and antimicrobial test results could suggests a single species probably from a common clone. Although *T. ptyseos* has not been reported in human disease in Nigeria, poor hygiene at the abattoir may predispose abattoir workers and meat consumers to possible human infection. To our knowledge, there is no information on previous isolation of this organism from clinical samples and/or food in Nigeria. The isolation and identification of *T. ptyseos* in fresh meat (beef) in this study as well as in other food sources as reported by authors in other parts of the world underscore the need for more studies especially on the pathogenic potentials of the organism.

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