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The first report of *Escherichia fergusonii* isolated from non-human primates, in Africa

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ABSTRACT

The aim of this study was to determine the resistance phenotypes of selected enteric bacteria isolated from non-human primates at a wildlife-human interface. Bacterial isolates from faecal samples of non-human primates at two wildlife rehabilitation centres in South Africa were screened for the presence of *Escherichia coli*. The biochemical characterisation of *E. coli* and *E. coli*-like bacteria revealed both adonitol positive and sorbitol negative strains – a unique characteristic of *Escherichia fergusonii* and *Escherichia coli* K99. Further tests were carried out to identify the isolates, namely growth on Simmons citrate agar supplemented with 2% adonitol and biochemical tests based on their ability to ferment cellobiose and D-arabitol. Antimicrobial sensitivity was determined with microbroth dilution tests employing microtitre plates with 21 different antimicrobial drugs. Molecular characterisation was done with a duplex polymerase chain reaction (PCR) assay that targeted the *yliE* and *EFER_1569* genes. *E. fergusonii* strains were confirmed by the presence of a 233 bp segment of the *yliE* gene and a 432 bp segment of the *EFER_1569* gene.

Twenty-three *E. coli*-like bacteria were confirmed as *E. fergusonii* based on the confirmatory tests and they were in 100% agreement. Approximately 87% of them were resistant to polymyxins B and E (colistin) as well as the carbapenem group with occasional resistance to amikacin.

This is the first reported isolation and identification of *E. fergusonii* strains in non-human primates. The findings point to *E. fergusonii* as a possible emerging pathogen of zoonotic importance.

1. Introduction

In the last four decades, descriptions of *Escherichia blattae* [5], *E. hermanii* [3], *E. vulneris* [4], *E. fergusonii* [7] and *E. albertii* [15] have been published as additions to the genus *Escherichia*. The recent identification of other species in this genus and genomic clades of *Escherichia coli* has been attributed to the improvement in diagnostic techniques [12]. As a result of the availability of an increasing variety of growth media and the introduction of PCR-based assays and whole genome sequencing, phenotypic similarities are not limiting factors anymore in differentiating between species of the genus *Escherichia*.

Escherichia fergusonii was proposed as a new species within the genus *Escherichia* and family *Enterobacteriaceae* in 1985 with a 64% similarity to *Escherichia coli* when analysed by means of DNA hybridisation [7]. This bacterial strain was formerly known as Enteric Group 10, due to its biochemically distinct nature compared to other species and bio-groups of the genus *Enterobacteriaceae*. DNA-DNA hybridisation to determine the relatedness of *E. fergusonii* strains to other species within the genus *Enterobacteriaceae* using ^{32}P-labeled DNA in 60 °C hydroxyapatite, showed 90–97% relatedness to the type strain (holotype) *E. fergusonii* ATCC 35469. In relation to other species, the closest was *Escherichia coli* with up to 64% similarity [7].

E. fergusonii was initially isolated from human clinical samples collected from the blood, urine, abdominal wounds and faeces of patients [7]. It has been isolated from gall bladder fluids of patients [10] and from food products such as montasio cheese [17]. It has also been isolated from faecal samples of goats [13], sheep [1], horses [25], turkeys, ostriches [14], chickens [20], cattle and pigs [7].

Aside the recent report of *E. fergusonii* isolations in fish in Egypt [11]; no other reports of *E. fergusonii* isolations have been documented from Africa either in humans or in animals. Since the majority of reported *E. fergusonii* isolations in other parts of the world were case reports [12], data on host species dynamics, reservoirs and transmission are currently unknown.

2. Literature review

In common with other *Escherichia* species, *E. fergusonii* is a rod-

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shaped, Gram-negative member of the family *Enterobacteriaceae*. It is a non-spore-forming, predominantly motile, peritrichous flagellated bacterium. It has a diameter of 0.8–1.5 μm and lengths from 2 to 5 μm. *E. fergusonii* grows optimally on growth media at 37–40 °C under aerobic conditions with variations between 21 and 45 °C [12,23].

Citrate adonitol agar has been reported to be an effective selective growth medium for isolating *E. fergusonii* from faecal samples [26]. This medium was first described in 1984 as a selective medium for the isolation of certain K99 *E. coli* strains based on their ability to ferment adonitol. Recent studies also employed the use of CHROMAgar Orientation media in isolating *Escherichia* species. *Escherichia coli* colonies appear pink on the growth medium whilst *E. fergusonii* colonies appear pink with brown discoloration surrounding the colonies [16].

Simmons et al. [24] described a novel PCR assay for the detection of *E. fergusonii* directly from caecal and cloacal samples of poultry without pre-enrichment. For that study, primers targeting specific genes, including *yliE* (encoding a conserved hypothetical protein of the cellulose synthase and regulator of cellulose synthase island), *EFER_1569* (encoding a hypothetical protein, putative transcriptional activator for multiple antibiotic resistance), and EFER_3126 (encoding a putative triphosphoribosyl-dephospho-coenzyme A [CoA]), were designed for the detection of *E. fergusonii* by conventional and real-time PCR methods.

A fluorescent dye-labeled probe for the enumeration of *E. fergusonii* cells was tested by *in situ* hybridisation and epifluorescence microscopy and found to be able to stain cells of *E. coli*, *Shigella* spp. and *E. fergusonii* [22]. The specificity of the probe is limited to the aforementioned species; having being tested on 169 other species.

These molecular techniques improved the sensitivity and speed of diagnosis of *E. fergusonii* infections. As an opportunistic pathogen, usually isolated from infections in which the causative pathogen is initially unknown, molecular detection speeds up the detection/confirmation processes considerably [23].

Earlier experiments on *E. fergusonii* strains recorded resistance to ampicillin, tetracycline and co-trimoxazole and susceptibility to cephalosporin and netilmicin [6].

Lagacé-Wiens et al. [16] also reported the presence of large quantities ($> 10^8$) colony-forming units per litre of urine (CFU/l) of extended-spectrum β-lactamase (ESBL)-producing *E. fergusonii* in a 76-year-old Caucasian woman presented to the emergency department of a community hospital. This report was the first case of a clinical isolate of multidrug-resistant *Escherichia fergusonii* expressing an extended-spectrum-β-lactamase (ESBL).

Ampicillin-resistant *E. fergusonii* isolates from farm animals were tested for extended-spectrum β-lactamase (ESBL) phenotypes by double disc diffusion tests using three indicator cephalosporins: cefotaxime, ceftazidime and cefoxitin, both alone and in combination with amoxicillin-clavulanic acid (AMC). PCR and sequencing of SHV, CTX-M, and TEM β-lactamase genes as described by Mulvey et al. [19] revealed the presence of TEM-1 and SHV-12 genes – the presence of the latter conferring the ESBL phenotype [16].

In another study on day-old-chick models [8], it was observed after genome analysis of an isolated *E. fergusonii* strain that the existence of several resistance genes to multiple classes of antibiotics was present in the strain, thus making the treatment of infection caused by such *E. fergusonii* strains difficult when using currently available antimicrobials [8].

The presence of multiple resistance genes in their genomes gives rise to multi-drug resistant *E. fergusonii* strains. Most *E. fergusonii* strains that are isolated from both humans and animals – in cases where general first-line drug treatments for bacterial infections fail – are multi-drug resistant [23,2].

3. Materials and methods

3.1. Study location and animals

Two wildlife rehabilitation centres located within 10 km of each other and approximately 30 km from the Kruger National Park served as study sites for this project. These centres were established to provide temporary sanctuary for injured and orphaned wildlife, rehabilitate them to a point of self-support and their ultimate release and introduction into nature, where they naturally belong. They rehabilitate and provide sanctuary to over 500 orphaned, injured, abused, ex-laboratory baboon and vervet monkey populations. In both centres, the non-human primates were classified into three groups and this influenced the sampling strategies.

The first group had regular human contact and were mostly orphaned juveniles that were fed, bathed and cuddled regularly. The second group of non-human primates had been weaned off frequent contact with humans with occasional contact when it was time to feed them. There was no cuddling or bathing of the animals in this group and they only came in contact with staff/volunteers during feeding lasting a maximum of 10 min, 3 times daily. The third group had previous human contact and lived in enclosures similar to their natural habitats with almost no human contact. They fed off natural shoots in their enclosures and food was only supplemented in harsh weather conditions when plants they feed on were not available.

3.2. Sample collection and processing

There were three major groups defined per rehabilitation centre for sampling purposes in line with the groups within the centres. Three hundred fresh faecal samples were obtained from vervets and baboons in sampling groups using sterile swabs immersed in Stuart's transport medium. The animals were anally swabbed early mornings between 6 and 7 am, for three days by staff at the centres. One-hundred-and-fifty (150) baboon faecal samples and fifty faecal samples from vervets were collected at one wildlife rehabilitation centre whilst another 100 faecal samples from vervets were collected at the second wildlife rehabilitation centre, in triplicates.

Sample size was random and representative of at least 25% of the total population.

3.3. Bacterial isolation

To isolate Gram-negative bacteria, the 300 faecal samples were inoculated on MacConkey agar under sterile conditions and incubated for 24 h at 37 °C. Suspect *E. coli* and *E. coli*-like isolates were then inoculated on Eosin Methylene Blue (EMB) agar.

3.4. Biochemical tests

Biochemical characterisation of *E. coli* and *E. coli*-like isolates were carried out using the Remel® RapID One panel strips (Remel, UK). Oxidase tests were done prior to the panel strip tests to ensure only oxidase-negative isolates were tested - as recommended by the manufacturer. Samples were transferred to inoculation fluids and then into wells imbibed with one reactive ingredient each and incubated at 37 °C for 4 h. The biochemical profiles were then determined using the Remel Eric system (Remel, UK) after incubation, based on colour changes in the inoculated wells - as indicated by the manufacturer.

Adonitol positive and sorbitol negative *E. coli*-like isolates were classified as suspected *E. fergusonii* strains and picked out for further testing. Two tests were carried out for further confirmation of *E. coli* and *E. coli*-like colonies, namely selective isolations on Simmons citrate agar supplemented with 2% adonitol [9] and biochemical tests based on their ability to ferment cellobiose and D-arabitol as described by Huys et al. [15].