Inhibition of Assemblage and Inactivation of Pilus Prevented Escherichia Coli Infection in Rat

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Abstract:- Escherichia coli has been the principal causative agent of urinary tract infections (UTI), forming about eighty percent (80%) of such cases. E. coli must attach itself to the host tissue with its pili in order to initiate infection. This study was an attempt to investigate the effect of inhibition of assemblage and inactivation of the pili of E coli in UTI infection.

Inhibition of assemblage of pili was attempted by culturing an infected mid-stream urine (MSU) on MacConkey agar containing 10mg/L inhibitor N-(4-chloro-phenyl)-2-5-(pyrrolidine-1-sulfonyl)-phenyl]-1(3,4)oxidiazol-2-ylsulfany1)-acetamide (AL1). The MSU was also cultured on plain MacConkey agar. Isolate from plain MacConkey agar was treated with inhibitor for pili inactivation trial. 0.2ml of 4.0 x 10⁷/ml bacterial suspension was administered intraperitoneally into sets of 12-week old rats. Infection was followed for 10 days, after which the rats were sacrificed. Urines from the rats were cultured on Mcc agar, bladders were excised and fixed for histological studies.

There were indications that inhibition of assemblage and inactivation of pili prevented infection in the rats, because there were no growth from the urines of the rats that were administered with isolates from AL1-treated MacConkey, while there was growth of E. coli from the urines of rats administered with isolates from plain MacConkey. Histological studies also showed normality in the bladders of rats that were administered with AL1-treated isolates of E. coli, whereas there were some tissue abnormalities in those that received AL1-free isolates.

Inhibition of assemblage of pili is a good strategy to prevent UTI by E. coli, and AL1 is an effective chemical pilus inhibitor.

Keywords: Escherichia coli, pili inhibition, pili inactivation, pili inhibitor (AL1), UTI.

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I. Introduction

Attachment of bacteria is a necessary step in the pathogenic process. Many pathogenic bacteria attach to a host cell before they can infect it. In order to prevent themselves being flushed out in the urine, Escherichia coli, and some other pilus-producing bacteria attach themselves with the aid of hair-like structures type 1 pili (1-8). Type 1 pili are peritrichously expressed filamentous surface structures ranging from a few micron to greater than 3 microns in length.

A chemical inhibitor, has been discovered that interferes with the essential steps in the assembly process of the pili.

The inhibitor, N-(4-chloro-phenyl)-2-5-(pyrrolidine-1-sulfonyl)-phenyl]-1(3,4)oxidiazol-2-ylsulfany1)-acetamide, simply called (AL1), is able to prevent production of pili by bacteria. The “naked” bacteria i.e bacteria without pili, can no longer be able to attach to their host cell.

This work focuses on the effect of AL1 on the virulence of Escherichia coli, the specie that causes about 80% of urinary tract infections (UTIs). (9-12), using available local tools.
II. Materials and Methods

2.1 Materials:
Pilus inhibitor (AL1) Molport-000-031-008 was procured from Molport company; MacConkey agar Biotek product; 12 week-old rats procured from the Animal house of Bowen University Teaching Hospital, Ogbomoso, Nigeria.

Ethical Clearance:-
The ethical review committees of Shalom Medical Centre, Ogbomoso and the animal ethics committee of Bowen University Teaching Hospital, Ogbomoso, Oyo State, Nigeria, approved the study. Written informed consent of the urine sample donor was obtained.

2.2 Methods
Midstream urine (MSU) was collected from a female patient attending Shalom Medical Centre, Ogbomoso, with urinary tract infection before the administration of chemotherapy.

Culture Media:
10mg/L AL1-McC, and plain McC agars were prepared.

Isolation and identification of organism:
MSU sample was cultured on both media, and incubated aerobically at 370 c for 24 hrs.
Biochemical tests confirmed the isolates to be E, coli

Animal inoculation:
A set of rat was inoculated intraperitoneally with 0.2ml/ml of 4.0 x 10^7/ml isolate suspension from AL1-McC
A control set was inoculated with 0.2ml sterile normal saline
A set with whole cell isolate from plain McCoy that has been exposed for 2 hours to AL1.
All animals were fed on pellets and sterile water for 10 days.
All animals were sacrificed by cervical dislocation 10 days post infection.
Urines from the animals were aspirated and cultured on plain McCoy agar. Incubation was at 370 c, aerobically for 24 hours.

Histological Studies:
Bladders were excised from animals that were inoculated with isolates from plain Mcc, and AL1-treated Mcc.
The tissues were preserved in 10% formal saline.
Tissue processing was done using automatic tissue processor Leica TP 1020.
Haematoxin and Eosin (H&E) technique was used for staining.

III. Results
Urines of rats that were administered with E. coli isolate from AL1-treated MacConkey. (Fig. 2 Column A), and the whole cell E. coli pre-treated with AL1, (fig 2D column), yielded no growth. No growth from the control (fig 2C).
Urines of rats that were administered with isolates from plain (AL1-free) MacConkey agar yielded pure growth of E. coli (fig 2 B column).
Histological studies showed some abnormalities in the bladders of rat that were administered with isolates from plain (AL1-free) MacConkey (fig 3 A). There were no such abnormalities in the bladder of rats that were administered with whole cell E. coli pre-treated with AL1 (fig 3B).
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Fig. 1: showing primary isolation of E. coli from patient’s urine sample

A. Isolate on plain McC agar
B. Isolate on AL1-McC agar

Fig. 2: Picture showing the results of urine cultures of rats administered with isolates:

A. Urine of rat administered with E. coli isolate from AL1-treated McC (No growth)
B. Urine of rat administered with E. coli isolate from plain McC showing growth of lactose-fermenting E. coli
C. Urine of control rat administered with normal saline (No growth)
D. Urine of rat administered with whole cell E. coli pre-treated with AL1 (No growth)
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Fig. 3A Photomicrograph showing stained bladder section of rat administered with E. coli isolate from plain McC.

Photomicrograph of a bladder section stained by Haematoxylin and Eosin showing poor architecture- there is moderate to severe thickening of the urothelium (white arrow), the muscularis propria under the urothelium appear mildly loosen (black arrow).

Fig. 3B Photomicrograph showing stained bladder section of rat administered with whole cell E. coli pre-treated with AL1.

Photomicrograph of bladder section stained by Haematoxylin and Eosin showing moderately normal architecture, the urothelium appear moderately normal, the muscularis propria under the urothelium is mildly loosen, and the general connective tissues appear normal.
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IV. Discussion

This work confirms the role of pili as a necessity to initiate UTI. Several researchers have reported this (13-20). Inhibition of assemblage & inactivation of pili prevented E. coli UTI in rat. AL1 was found to be effective in the inhibition of assemblage and inactivation of pili in E. coli. This has also been established by other workers (12).

Some strains of E. coli, the extended-spectrum beta-lactamase (ESBL) are fast emerging. They are resistant to the conventional antibiotics, such of ciprofloxacin, trimethoprim-sulfamexazole (9, 21). It has been reported that extraintestinal pathogenic E. coli (EXPEC) also cause UTI in humans; chicken has been identified as reservoirs of extraintestinal pathogenic E coli that causes UTI in humans (22). Similarly food – borne origins of E. coli causes extraintestinal infection in humans. (23).

The adherence of E. coli to tissues with the pili promotes pathological change especially in the bladder, as reported by many workers (12, 24-26). The results of the histological studies in this study show such changes (Fig. 3A).

Pili are essentially a very potent virulent factor for the pathogenesis of E. coli, a bacterium incriminated in various infections in human, beyond UTI. Without pilus, attachment to host cell is not achievable, hence infection is prevented. Chemical inhibition of pili assemblage is disarming the bacteria rather than destroy, unlike the conventional antibiotics that destroy the pathogenic bacteria, a process that usually destroys beneficial commensals, resulting in development and spread of resistance bacteria. The strategy of pili inhibition has good benefits over the use of antibiotics, and the chemical could become new drug on the shelf (12). In this study, AL1 did not prevent the primary isolation of E. coli from the infected human urine used. (fig 1B). This implies that AL1 only disarms the organism by inhibiting pili assembly. Similarly, whole cell E. coli treated with AL1 2hrs before injected into the rats brought about inactivation of pili, hence there was no attachment to the bladder. (fig 2D).

With the rate of emergence of antibiotic – resistant strains of E. coli, there is the need for new approach to combat E. coli infections.

In vivo suitability of AL1 for chemotherapy against pili – producing Gram negative bacteria should be investigated.

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