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In vitro adherence of antibiotic-resistant *Escherichia coli* to biomaterial surfaces: Effect of conditioning film

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Abstract

In our recent study of patients attending a government hospital and a private medical laboratory in the Nigerian city of Zaria, we not only reported an increase in antibiotic resistance in urinary isolates of *Escherichia coli* but also the fact that the resistant isolates originated from an environment in which antibiotics were frequently used. The implication of the resistance on the adherence characteristics of these isolates is the main objective of this study. Using a laboratory model, we assessed the in vitro adherence to biologically inert surfaces of five representative antibiotic-resistant isolates with a view to finding any possible relationship between their resistance and adherence. Bacteria adhered poorly to the surfaces. Adhesion was enhanced, reduced or uninfluenced by conditioning the adhering surface with urine or serum depending on the adhering bacteria or type of biomaterials. There was no apparent relationship between the antibiotic resistance of these isolates and their adherence. Our observations suggest that the risk of biomaterial-associated infections is determined by both the adhering bacteria and type of biomaterial.

Key words: Adherence, biomaterials, *Escherichia coli*, antibiotic-resistant

Introduction

Infections of the urinary tract are among the most common infectious diseases in human, possibly because the urinary tract is in direct communication with the exterior (1). They are therefore, an important cause of morbidity and mortality in both adults and children (2). *Escherichia coli* remain one of the primary causes of acute cases of uncomplicated urinary tract infections (3-5).

Medical prostheses such as urinary catheter are increasingly being used in the practice of medicine. Their use however, may be associated with certain complications, the commonest being microbial infections (6, 7) resulting from adhesion of bacteria (particularly those resistant to antibiotics) (8), to the smooth surface of these devices. Stamm et al. (9) have also reported that as much as 35% of all nosocomial urinary tract infections due to Gram-negative bacilli were due to bacteria adhering to catheter. Colonization of the outer lumen of the catheter by microorganisms is usually the result of the catheter's proximity to skin flora. Colonization of the inner lumen of catheters (specifically by gram-negative rods) may be the result of a break in aseptic handling of the device prior to insertion or of the exposure of the end connectors to water, soil, or contaminated surfaces. Both gram-positive and gram-negative bacteria have been isolated from biofilms on biomaterials (10-12).

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We reported recently (13) an increase in antibiotic resistance in urinary isolates of *Escherichia coli* in the Nigerian city of Zaria. The implication of the resistance on the physicochemical virulence characteristics of the isolates is the main objective of this study. Using a laboratory model, we evaluated the in vitro adherence characteristics of five representative isolates (selected to reflect the different resistance patterns observed) with a view to finding any possible relationship between their resistance and adherence. In addition, the effect of urine- and serum-conditioning films on the adherence was investigated in a laboratory model.

Materials and methods

Bacterial isolates

Five clinical isolates (selected to reflect the different resistant patterns observed) were maintained at 4°C on tryptic soy agar (Merck Ltd., Germany) and grown at 37°C in tryptic soy broth (Merck Ltd., Germany) overnight each timebefore use. A standard strain *(Escherichia coli* NCTC 10418) was similarly treated as the test isolates, and used where necessary. Table 1 lists the characteristics of the isolates.

Table 1: Characteristics of the isolates[a]

Test isolates	Resistance pattern	Ampicillin MIC[b] (g/ml)	Multiple antibiotic resistance (MAR) indices[c]	-lactamase production
Ec164	ATC_1G	> 10000	0.50	+
Ec191	ATC_1GNaO_f	5000	0.75	+
Ec206	ATC_1	10000	0.50	-
Ec140	AT	1250	0.25	+
Ec219	ATC_1Na	5000	0.50	+

[a]From our recent study (Ngwai et al., [13]) ; [b]MIC, minimum inhibitory concentration; [c]Calculated by dividing number of antibiotics resistant/number of antibiotics examined; A = ampicillin; T = tetracycline; C_1 = co-trimoxazole; G = gentamicin; Na = nalidixic acid; O_f = ofloxacin

Conditioning of biomaterials

The method of Onaolapo & Salami (14) was used to condition the inert materials. Briefly, inert materials were properly washed with distilled water and sterilized by autoclaving. Urine or serum from healthy volunteer was filtered through Millipore membrane (0.22 μm) into a sterile beaker under laminar flow. The inert materials were then immersed into either, removed after 30 min and dried under the laminar flow resulting in urine- or serum- film coat on the surfaces of the inert materials.

Microbial adhesion to hydrocarbon (MATH)

This was determined based as described by Flint et al. (15). Briefly, each isolate was grown overnight at 37°C in 30 ml of tryptic soy broth in a water bath. Cells were then harvested by centrifugation (3000 rpm x 10 min) and re-suspended in sterile de-ionized water to an absorbance at 600 nm (A_{600}) of 1.2-1.6 (A_i) using a UV-Visible Spectrophotometer (Beckman Inc., U.S.A). 3.0 ml of each of the cell suspension was added to 3.0 ml of xylene (BDH Chemicals Ltd., England) in separate tubes and mixed briefly on a vortex mixer. These mixtures were left for 15 min at ambient temperature to allow equilibration to occur. They were subsequently mixed vigorously by vortex for 2 min at ambient temperature and

afterwards allowed to stand for 20 min to allow phase separation. The A_{600} of the aqueous phase after phase separation (A_f) was henceforth measured. The percent (%) hydrophobicity of the cell surfaces was then calculated using the relationship: $(1-A_f/A_i) \times 100$. The experiment was repeated and results presented here are average of triplicate determinations.

Microbial adhesion to inert surface (MATIS)

Adherence to urinary catheter (10 mm x 8 mm x 4 mm), Dacron intravenous (I.V.) catheter (27 mm x 10 mm) and glass (10 mm x 20 mm) surfaces, both coated and uncoated, was examined as described previously (14, 16). Briefly, 30 ml of tryptic soy broth was transferred into six conical flasks containing two pieces each of the inert materials and sterilized by autoclaving. After cooling, each flask was then inoculated with a different isolate and grown in a water bath maintained at 37°C for 24 h. The inert materials were then transferred aseptically into 10 ml of sterile normal saline in universal bottles and rinsed to remove loosely adhered cells. Each piece of the inert material was then placed individually in universal bottles containing 9.9 ml of sterile normal saline (this gives a 1:100 dilution approximately) and vigorously mixed by vortex for 2 min to dislodge adhering cells. The dislodged cells were then counted on nutrient agar (Lab M Diagnostic Products, England) plates after appropriate dilution in sterile normal saline. The experiment was repeated twice and average of results taken. 'Control' counts were also determined for the isolates. The percent (%) adhesion was then calculated for each isolate and standard strain using the relation: (No. cells adhering to inert material/control count) x 100

Results

Cell surface hydrophobicity

The isolates and Type strain differed from each other in their relative cell surface hydrophobicity (Fig. 1).

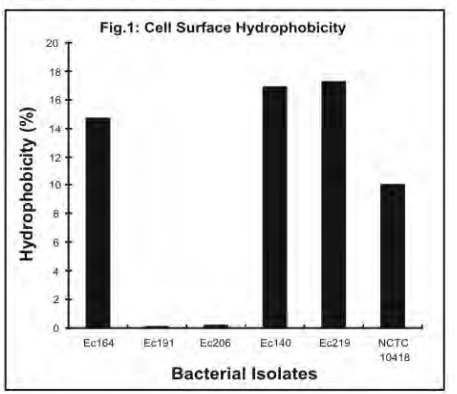
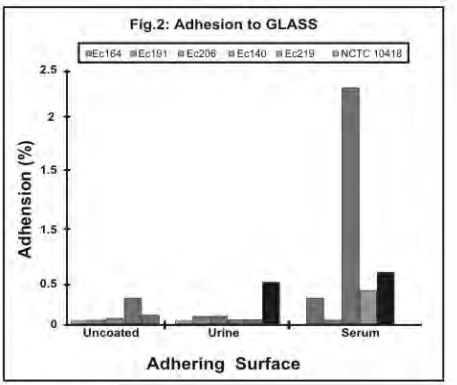


Fig. 1: MATH assessment of the cell surface hydrophobicity of antibiotic-resistant *Escherichia coli* isolates. NCTC 10418: *Escherichia coli,* National Collection Type Culture; hydrophobicity (%) = $(1-A_f/A_i) \times 100$, where A_i = initial absorbance of the bacterial suspension and A_f = absorbance of aqueous phase after separation of xylene.

Adherence to glass

All the isolates adhere to uncoated glass to different extents that do not relate to their cell surface hydrophobicity (Fig. 2). Coating of glass surface with urine significantly enhanced the adhesion of only the drug sensitive Type strain; adhesion of one of the resistant isolates (Ec219) was drastically reduced by the urine coating. With the exception of the â-lactamase-negative isolate (Ec206), the adhesion of all other isolates was significantly enhanced by serum-coating of the glass surface.

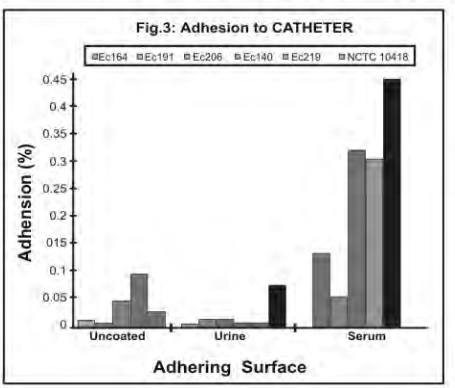
Fig. 2: Adherence of antibiotic-resistant *Escherichia coli* to glass. NCTC 10418: *Escherichia coli*, National Collection Type Culture; adhesion (%) = (No. of cells which adhered/control) x 100.



Adherence to catheter

With the exception of the â-lactamase-negative isolate (Ec206), whose adhesion was reduced by urine-coating, the adhesion of all other isolates and Type strain to catheter was greatly increased by both urine- and serum-coating of the catheter surface; the effect of serum was more than that of urine (Fig. 3). It also appears that the catheter was a better surface for adhesion compared with glass and cannula.

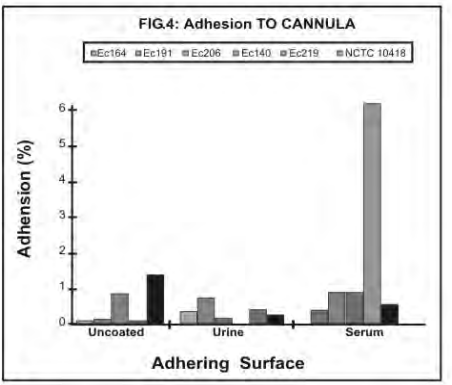
Fig. 3: Adherence of antibiotic-resistant *Escherichia coli* to urinary catheter. NCTC 10418: *Escherichia coli*, National Collection Type Culture; adhesion (%) = (No. of cells which adhered/control) x 100.



Adherence to cannula

The conditioning of cannula surface by urine and serum enhanced the adhesion of the test isolates except in the following two cases: Ec140 and Type strain (NCTC 10418), where both urine- and serum-coating reduced adhesion (Fig. 4).

Fig. 4: Adherence of antibiotic-resistant *Escherichia coli* to Dacron intravenous (I.V.) cannula. NCTC 10418: *Escherichia coli*, National Collection Type Culture; adhesion (%) = (No. of cells which adhered/control) x 100.



Discussion

The fundamental mechanisms governing bacterial adhesion, whether to epithelial cells or non-living surfaces, are still not fully understood. It is thought to be a complex process, involving passive mechanisms (van der Waals' attractive forces, electrostatic interactions, hydrophobic and steric forces) and a more active mechanism- production of polymers by the bacteria (17).

The isolates we examined showed relatively different surface hydrophobicity by the MATH method. This may be a reflection of the relative differences in cell surface components since cell surface hydrophobicity can be influenced by the expression of surface components (18, 19); and the hydrocarbon in MATH assay acts by interaction with the inner core region of the LPS (20), or with the membrane phospholipids (21). We used xylene as the hydrocarbon phase because it is more hydrophobic and can permit the detection of relatively less hydrophobic cells better than aliphatic hydrocarbons like hexadecane (22). Xylene is also less negatively charged when in contact with aqueous solutions at pH 7.0, including Phosphate-buffered saline (PBS) than aliphatic hydrocarbons such as hexadecane, n-octane, etc (23).

The low ability of the isolates to adhere to the inert surfaces of different intrinsic hydrophobicity may be accounted for by the isolates' low levels of surface hydrophobicity. This is because cell surface hydrophobicity and charge have also been shown previously to be the most important determinants of adhesion (24-26).

The enhanced adhesion by conditioning of the inert surfaces with urine or serum (to simulate a clinical situation of prostheses' use) is in agreement with previous findings with other

Gram-negative bacteria (14, 27). Material surfaces adsorb proteins or other organic materials when exposed in a fluid environment such as urine and serum. As observed in this study, these organic coatings, or conditioning films, have been shown to alter the material surface properties and affect microbial attachment (28, 29).

â-lactamase is protein in nature, and its release into a fluid environment containing biomaterials is expected to alter the surface properties and affect microbial attachment. This could explain why the adhesions of the â-lactamase-negative isolate on all surfaces, whether modified or not, was generally poor.

Overall, there was no apparent relationship between the antibiotic resistance of these isolates and their adherence. Our observations also suggest that the risk of biomaterial-associated infections is determined by both the adhering bacteria and type of biomaterial.

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References

1. El-Bashier, A.M. (1991). Bacteriuria, incidence, causative microorganisms and susceptibility pattern at Quatif Central Hospital. *Ann. Saudi Med.* 11: 429-434.
2. Gerald, L.M., Douglas, R.G. and Bennette, J.E. (1990). *Principles and Practice of Infectious Diseases* 3rd ed. Churchill Livingstone, New York. pp. 582-590.
3. Gruneberg, R.N. (1984). Antibiotic sensitivities of urinary pathogens between 1971-1982. *J. Antimicrob. Chemother.* 14: 17-23.
4. Lamikanra, A. and Ndep, R.B. (1993). The incidence of multiple antibiotic resistances in urinary tract pathogens isolated from Nigerian patients. *Nig. J. Pharm.* 24: 28-30.
5. Ekweozor, C.C. and Onyemenem, T.N. (1996). Urinary tract infections in Ibadan: causative organisms and anti-microbial sensitivity patterns. *Afr. J. Med. Med. Sci.* 25: 165-169.
6. Iran, L.P., Chio, S.H. and Ventureyra, E.C.C. (1980). Complications of ventriculoatrial and ventriculoperitoneal shunts in a new children's hospital. *Can. J. Surg.* 23: 566-568.
7. Odio, C., McGracken, G.M. and Nelson, J.D. (1984). CSF shunts' infections in paediatrics: a seven-year experience. *Amer. J. Dis. Child.* 138:1103-1118.
8. Bayston, R. and Penny, S.R. (1972). Excessive production of mucoid substance in *Staphylococcus* SIIA: a possible factor in colonization of@Holter shunts. *Dev. Med. Child. Neurol.* 27(Suppl.): 25-28.
9. Stamm, W.E., Martin, A.M. and Bennet, J.V. (1977). Epidemiology of nosocomial infections due to Gram-negative bacilli: aspects relevant to development and use of vaccines. *J. Infect. Dis.* 136 (Suppl.): 151-160.
10. Peters, G., Locei, R. and Pulverer, G. (1982). Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. *J. Infect. Dis.* 146: 479-482.
11. Nickel, J.C., Ruseska, I., Wright, J.B. and Costerton, J.W. (1985). Tobramycin resistance of *Pseudomonas aeruginosa* cells growing as a biofilm on urinary catheter material. *Antimicrob. Agents Chemother.* 27: 619-624.
12. Marrie, T.J. and Costerton, J.W. (1984). Morphology of bacteria attached to cardiac pacemaker leads and power packs. *J. Clin. Microbiol.* 19: 911-914.
13. Ngwai, Y.B., Onaolapo, J.A., Ehinmidu, J.O., Ibrahim, K. and Olutimayin, G. (2005). Frequency of Ampicillin resistance amongst uropathogenic strains of *Escherichia coli* isolated from patients with suspected urinary tract infections in Zaria. *Nig. J. Pharm. Sci.* 6: 26-32.
14. Onaolapo, J.A. and Salami, J.O. (1995). Effect of sub-minimum inhibitory concentration of Ceftriaxone on the adherence of *Pseudomonas aeruginosa* to inert surfaces in an experimental model. *Afr. J. Med. Med Sci.*, 24: 275-281.
15. Flint, S.H., Brooks, J.D. and Bremer, P.J. (1997). The influence of cell surface properties of the

thermophilic streptococci on attachment to stainless steel. *J. Appl. Microbiol.* 83: 508-517.
16. Onaolapo, J.A., Nuhu, Z.S. and Olurinola, P.F. (1997). Comparative studies on the surface properties of three *Escherichia coli* isolates. ***B**iomed. **Lett.*** 55: 211-219.
17. Oliveira, D.R. (1992). Physico-chemical aspect of adhesion. *In: Biofilms: science and technology.* Melo, L.F., Bott, T.R., Fletcher, M. and Capdeville, B. (eds.). pp. 45-48, Academic Press, Dordrecht-Kluwer.
18. Ofek, I., Whitnack, E. and Beachey EH (1983). Hydrophobic Interactions of Group A streptococci with Hexadecane Droplets. *J. Bacteriol.* 154: 139-45.
19. Rosenberg, E., Kaplan, N., Pines, O., Rosenberg, M. and Gutnick, D. (1983). Capsular polysaccharides interfere with adherence of *Acinetobacter calcoaceticus* to hydrocarbons. *FEMS Microbiol. Lett.* 17: 157-160.
20. Rosenberg, M., Gutnick, D. and Rosenberg, E. (1980). Adherence of bacteria to hydrocarbon: a simple method of measuring cell-surface hydrophobicity. *FEMS Microbiol. Lett.* 9: 29-33.
21. Rosenberg, M., Rotten, S. and Rosenberg, E. (1982). Cell-surface hydrophobicity of smooth and rough *Proteus mirabilis* strains as determined by adherence to hydrocarbon. *FEMS Microbiol. Lett.* 13: 167-169.
22. Kjelleberg, S. and Hermansson, M. (1984). Starvation-Induced Effects on Bacterial Surface Characteristics. *Appl. Environ. Microbiol.* 48: 497-503.
23. Busscher, H.J., van de Belt-Gritter, B. and van der Mei, HC. (1995). Implications of microbial adhesion to hydrocarbons for evaluating cell surface hydrophobicity. 1. Zeta potentials of hydrocarbon droplets. *Colloids Surf. **B*** 5: 111-116.
24. Dahlback, B., Hermanson, M., Kjelleberg, S. and Norkrans, B. (1981). The hydrophobicity of bacteria: an important factor in their initial adhesion at the air-water interfaces. *Arch. Microbiol.* 128: 267-270.
25. Criado, M.T., Ferreiros, C.M. and Sainz, V. (1985). Adherence and hydrophobicity in *Neisseria meningitidis* and their relationship with surface charge. *Med. Microbiol. Immunol.* 74: 151-156.
26. Hood, S.K. and Zottola, E.A. (1995). Biofilms in food processing. *Food Control* 6: 9-18.
27. Vaudaux, P. E., Lew D. P. and Waldvogel F. A. (1994). Host factors predisposing to and influencing therapy of foreign body infections. In: *Infections associated with indwelling medical devices* 2nd ed. Bisno A.L. and Waldvogel F.A. (ed.). pp. 1-29, ASM Press, Washington, D.C.
28. Murga, R., Miller, J.M. and Donlan, R.M. (2001). Biofilm Formation by Gram-Negative Bacteria on Central Venous Catheter Connectors: Effect of Conditioning Films in a Laboratory Model. *J. Clin. Microbiol.* 39: 2294-2297.
29. Characklis, W.G., McFeters G.A. and Marshall K.C. (1990). Physiological ecology in biofilm systems. In: *Biofilms*. Characklis W.G. and Marshall K.C. (ed.). pp. 341-394, John Wiley & Sons, Inc., New York.