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# Hydrophobic response of *Escherichia coli* exposed to subminimal inhibitory concentrations of ampicillin and chloramphenicol

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## Summary

Hydrophobicity generally increased as the cells passed from lag to exponential phases of growth and declined in the stationary phase. All concentrations of ampicillin used increased hydrophobicity, although still subject to effect of phase of growth. Chloramphenicol caused decline in hydrophobicity. Combination of the two antibiotics gave a concentration dependent balance of the two forces observed. Protein synthesis inhibition may render cells resistant to phagocytic uptake by lowering surface hydrophobicity. This phenomenon is probably involved in cases of therapeutic failures, persistent of recurrent infections. This is a further indication of the undesirability of antibiotic abuse.

## Résumé

Le taux d'hydrophobie augmente en général lorsque les cellules passent d'une phase de croissance à retardement à une phase de croissance exponentielle, et baisse au cours d'une phase de croissance stationnaire. Le taux d'hydrophobie augmente pour toutes les concentrations d'ampicilline utilisées, bien que demeurant sujet à l'effet des phases de croissance. L'utilisation du chlorampénicol entraîne une baisse du taux d'hydrophobie. L'association des deux antibiotiques résulte en un équilibre des deux forces observées qui dépend de la concentration. L'inhibition de la synthèse protéique peut entraîner une résistance des cellules à la phagocytose, par l'abaissement du taux d'hydrophobie superficielle. Ce phénomène est probablement impliqué dans les cas d'échecs thérapeutiques, d'infections persistantes ou périodiques. Ceci est un indice supplémentaire quant à l'inopportunité des abus des antibiotiques.

## Introduction

Hydrophobicity is an index of the hydrophobic nature of bacterial cell surface. The nature of the cell surface is significant considering the fact that interaction of pathogenic bacterial with cells of the host results from the balance of several attractive and repulsive forces operating between the complex surface components of procaryotes and eucaryotes[1].

Hydrophobic interactions have been implicated in adherence of bacteria to phagocytes[2,3], host tissues during colonisation and in cell aggregation[4].

As a surface phenomenon, bacterial hydrophobicity has been associated with various cell envelope components like: proteins[5-7], lipoteichoic acid[8-10], glycolipids[6] and phospholipids[11].

The nature of the growth environment is known to influence bacterial hydrophobicity. For instance, diphenylamine has been reported to inhibit the synthesis of polysaccharide component of surface lipopolysaccharide (LPS)[12]. Also polymyxin B has been reported to inactivate the LPS[13,14]. This inhibition of polysaccharide synthesis or LPS inactivation may result in an increase in surface hydrophobicity due to reduced shield from the diminuted hydrophilic LPS according to Rosenberg *et al*[15].

Use of sub-minimal inhibitory concentrations (Sub-MICs) of antibiotics in this work was prompted by the rampant incidence of antibiotic abuse resulting from self medication and therapeutic non-compliance in developing countries with particular reference to the Nigerian situation. This abuse, more often than not gives low blood levels of the antibiotics.

## Materials and methods

The organism used, *E. coli* 7 NCTC 9001 was grown in Nutrient broth (Oxoid and pH 7.4). The antibiotics used (obtained as formulated products) were: Ampicillin, Laboratory Torlan, S.A. Cerdanyola

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(Spain) brand and Chloramphenicol, Lab. Valles Mestre/Farm S.A. Quintanilla (Spain) brand. They were obtained from registered pharmacies in Ibadan. Concentrated stock solution of each antibiotic was prepared and divided into aliquots and kept frozen until required. Each aliquot once defrozen is not refrozen.

*M.I.C. determination*

The M.I.C. of each antibiotic was determined using the broth dilution method of Waterworth[16].

*Hydrophobicity measurements*

Cells from two 10ml overnight cultures were harvested by centrifugation at 3000 revolutions per minute for 15 minutes. The cells were washed once with nutrient broth at 37°C and resuspended in 2.5 ml of warm nutrient broth. Two millilitres of the cell suspension was then used to inoculate 100 ml of nutrient broth in a 250 ml conical flask, at 37°C. Four of such flasks were prepared and to one each of 3 flasks was added sub-MICs chosen to present a range of possible low antibiotic concentrations that could result from the haphazard manner of antibiotic abuse in the society. The added concentrations were: 1/2, 1/4 and 1/8 MIC of antibiotic (or antibiotics in the case of antibiotic combination) respectively. The flasks were then incubated at 37°C under static conditions (to approximate natural situation) and sampled after 4, 8 and 12 hours of exposure. Cell surface hydrophobicity was then measured using the bacterial adherence to hydrocarbon method of Rosenberg[17], using *n*- Hexane. The absorbance was taken at 430 nm using Spectronic 21 (BAUSCH & LOMB) Spectrophotometer.

## Results

*M.I.C.*

The M.I.Cs of the two antibiotics against the organism were Ampicillin (1.25µg/ml) and Chloramphenicol (50µg/ml).

*Hydrophobicity*

The cell surface hydrophobicity measurements of the cells treated with 1/2, 1/4 and 1/8 MIC are as presented in Figures 1:(A), (B) and (C) respectively. The per cent hyrophobicity index indicates the fraction of the cells in the bulk aqueous phase left after interaction with the hydrocarbon.

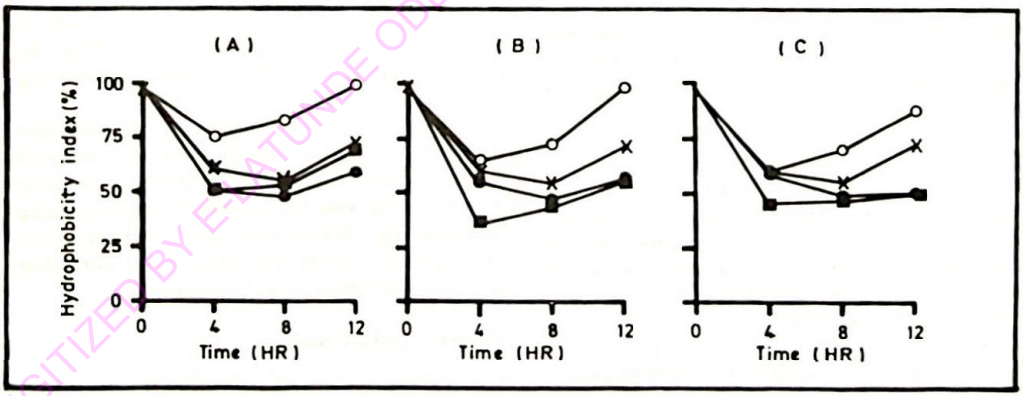


**Fig. 1:** Effects of ampicillin, chloramphenicol and time of exposure on cell surface hydrophobicity in *E. coli*. % Hydrophobicity index is the fraction of the total number of cells in the bulk aqueous phase left after interaction with hydrocarbon. (X) Control, ( • ) Ampicillin, (O) Chloramphenicol, (▬) Ampicillin + Chloramphenicol, at (A) 1/2 MIC (B) 1/4 MIC and (C) 1/8 MIC.

It was observed that hydrophobicity generally increased as the cells passed from the lag phase to exponential phase of growth. It again declined as the cells entered the stationary phase of growth.

At all concentrations used, ampicillin increased hydrophobicity though subject to the observed effect

of phase of growth. Chloramphenicol caused a decrease in hydrophobicity after an initial increase that is most likely due to the effect of the exponential phase.

On combination of the two agents, a concentration - dependent balance of the two forces was observed (Fig 1: A and B).

## Discussion

This study has shown that ampicillin acts on the cell envelope to increase cell surface hydrophobicity (CSH). This effect is probably due to exposition of more of its lipophilic components as suggested by Kadurugamuwa *et al*[18] for some cephalosporins on *Klebsiella pneumoniae* 327. With chloramphenicol there seems to be a reduction in the lipophilic component of the cell envelope. This is not suprising since chloramphenicol acts by inhibiting protein synthesis and proteins have already been associated with CSH[5-7]. The decrease in CSH, observed when cells were treated with chloramphenicol suggests that the ribosomal activities going on in the cytoplasm in the presence of sublethal concentrations of antibiotics have bearing on the CSH of the bacteria. CSH has been associated with bacteria cells susceptibility to phagocytic uptake[18,19] which is normally followed by killing and digestion. On the basis of established relationship between hydrophobicity and phagocytosis[2,3,19], it can be inferred that a fall or decrease in CSH may confer a measure of resistance to phagocytic uptake on the cell. This suggests that the use of bacteriostatic protein inhibitors should be discouraged. Similarly, the abuse of even potent and bactericidal protein inhibitors should also be discouraged, since the resulting sub MIC doses would have the same effect as bacteriostatic agents. Hence, some of the cases of resistance or therapeutic failure, persistent or recurrent infections might not be unconnected with the phenomenon of the antiphagocytic effect of a decrease in CSH consequent upon protein synthesis inhibition.

In agreement with some of the earlier published reports[6,8,11] it is obvious that other cell envelope components contribute to CSH such as were exposed by ampicillin, an effect that was not abolished by chloramphenicol.

The relative further increase in CSH by ampicillin in the presence of 1/4 and 1/8 MIC chloramphenicol (Figures 1B and 1C) might be due to improved penetration by ampicillin. Figures 1b and 1c however show a stronger chloramphenicol influence after a balance of the two forces.

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