COLORIMETRIC SERUM RESISTANCE ASSAY OF DRUG RESISTANT *E. COLI* STRAINS

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IT is well known that serum has bactericidal activity for most of the gram negative bacteria and it seems likely that this activity may represent an important host defence mechanism against infections. Although non-pathogenic bacteria and non-invasive pathogens are usually more sensitive to the lethal activity of serum but invasive pathogens are frequently resistant[1-3]. These findings and studies with experimental animal models[4] indicate that the serum resistance of invasive bacteria is important for their pathogenicity[1,5].

Considerable interest has developed in recent years in the mechanism whereby bacteria may be resistant to serum[2,6]. In the present study serum sensitivity of *E. coli* strains has been determined colorimetrically.

Strains of *E. coli* (310 in number) were used in this study. Their source of isolation and clinical conditions from which they have been isolated have been presented in table 1, their antibiotic sensitivity pattern has already been conducted in this laboratoty[7,8].

TABLE 1

Source of isolation of E. coli strains

Source	Clinical conditions	No. of strains
Human	Urinary tract infections	103
	Gastrointestinal tract infections	49
Animal		
Buffalo	Gastrointestinal tract infection	19
Calves	Gastrointestinal tract infection	58
Goats	Gastrointestinal tract infection	3
Sheep	Gastrointestinal tract infection	11
Equines	Utero-genital tract infections	48
Poultry	Septicaemia	19
	Total	310

For serum sensitivity assay, overnight grown culture, were washed with 0.14 M NaCl and resuspended in 3 ml of 0.14 M NaCl solution. About 0.5 ml of this suspension was exposed to 1% freshly obtained normal rabbit serum in peptone glucose broth containing the pH indicator bromothymol blue (pH range 6.0–7.6) and incubated at 37° C for overnight. Colour change is monitored spectrophotometrically at OD 500 against the normal control tube. The basis of colorimetric change in peptone glucose broth is due to the hydrolysis of glucose. If the bacteria are killed by normal rabbit serum, the medium remains green due to the presence of bromothymole blue indicator, whereas if they survive and grow, they produce acid from the glucose and the medium turns yellow.

TABLE 2

Serum sensitive and serum resistant strains of E. coli and their antibiotic sensitivity patterns

	Antibiotic resistance pattern			
	Serum sensitive strains		Serum resistant strains	
Resistance pattern	Total no. of strains	%	Total no. of strains	%
Single	11	18.7	54	23.7
Double	11	18.7	40	17.6
Triple	7	11.9	39	17.1
Quadruple	3	5.1	26	11.4
Quintuple	1	1.7	38	15.6
Multiple	4	6.8	30	14.2

Among 310 strains only 59 (19%) were found sensitive to serum and the remaining 251 (80.9%) were serum resistant. Out of these 251 serum-resistant strains, only 24 (9.5%) were those which were antibiotic sensitive, the remaining 227 (90.4%) strains were these, having both serum resistance and antibiotic resistance characters together. These findings agree with previous reports that some plasmids when present, substantially increase serum resistance levels of their host bacteria[9-11]. Further serum resistance can be encoded by antibiotic resistance plasmids and is consistent with the increasing incidence of antibiotic resistance plasmids in pathogenic bacteria.

The rapid colorimetric serum resistance assay described here will enable the large scale screening of bacterial isolates from clinical specimens for their serum resistance/sensitivity and this property will further facilitate the epidemiological investigations during Enteropathogenic *E. coli* outbreaks.

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β-RESORCYLALDEHYDE AS A CHROMATOGRAPHIC SPRAY REAGENT FOR METAL IONS

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β -RESORCYLALDEHYDE or 2,4-dihydroxybenzaldehyde has been employed as a specific chromatographic colour reagent for amino acids[1], and some of its derivatives have been shown to possess antibacterial[2] and antimalarial[3] activity. It has also been employed as a spectrophotometric reagent for the determination[4] of iron(III). In the present study it has been found to give colour reactions with 32 metal ions on a paper chromatogram in the visible and UV light. The colour reactions are particularly intense with transition metal ions: Ti(IV), V(V), Mn(II), Fe(II), Fe(III), Co(II), Ni(II), Cu(II), Ce(III) and U(VI). The sensitivity limits of detection of these metal ions with the reagent have been determined. The compound can therefore be employed as a chromatographic spray reagent for the identification of metal ions in macro and micro quantities.

β -Resorcylaldehyde was prepared and purified, m.p. 135°C (Lit. 135°-136°C) by the usual procedure[5].

Metal ion aqueous solutions of appropriate concentrations were prepared from AnalaR grade soluble salts of the respective metals.

Ascending paper chromatography, using Whatman No. 3 filter paper, was used for the development of chromatograms. *n*-Butanol: 6N HCl (1:1 V/V) solvent system was employed in all cases. The chromatograms were developed at room temperature (≈20° C) under optimum conditions of development. They were dried, exposed to ammonia and then sprayed with the reagent solution (0.5%) in 95% ethanol. The chromatograms were scanned in visible and UV light.

The colour reactions given by the 32 metal ions in the visible and UV light are listed in table 1. Table 2 shows the sensitivity limits with reference to the reagent and the corresponding values with other reagents wherever cited in the literature.

TABLE 1

Colour reactions of the metal ions in visible and UV light

ygr = yellow grey; ly = light yellow; y = yellow, gr = grey;
lgr = light grey; c = chocolate; yg = yellow green; fty = faint yellow;
brr = brick red; fbl = fluorescent blue; blw = blue white;
fw = fluorescent white; b = brown; dv = dark violet; v = violet;
lbl = light blue; lb = light brown; vgr = violet grey; db = dark brown;
yw = yellow white; dg = dark green.

M^{n+} (visible, UV)
Ag^{+}(—, lbl), Al^{3+}(ly, fw), As^{3+}(—, yw), Ba^{2+}(ly, dg), Be^{2+}(ygr, fbl), Ca^{2+}(—, blw), Ce^{3+}(lgr, vgr), Ce^{4+}(lgr, gr), Co^{2+}(lgr, vgr), Cr^{3+}(ly, lb), Cs (—, yg), Cu^{2+}(yg, db), Fe^{2+}(c, dv), Fe^{3+}(c, dv), Hg^{2+}(—, lbl), La^{3+}(ly, yg), Mg^{2+}(—, blw), Mn^{2+}(lgr, dv), Nd^{3+}(fty, brr), Ni^{2+}(lgr, vgr), Pr^{3+}(fty, brr), Rb (—, yg), Sb^{3+}(—, yw), Sn^{2+}(—, blw), Th^{4+}(ly, db), Ti^{4+}(y, b), U^{6+}(brr, db), V^{4+}(gr, dv), V^{5+}(lgr, v), Y^{3-}(fty, lbl), Zn^{2+}(—, fbl), Zr^{4+}(fty, b).