Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *88* (1): 135-140, jan./mar. 1993

USE OF A SELECTIVE MEDIUM WITH POTASSIUM TELLURITE TO FOLLOW INTESTINAL COLONIZATION OF HOSPITALIZED PATIENTS BY DRUG-RESISTANT *ENTEROBACTERIACEAE*

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Nosocomial infections are a relevant factor in complicating the recovery of patients interned for even minor causes. In a attempt to determine their origin it is crucial to consider that their origin is of an endogenous nature.

Looking for an acessible expression of intestinal colonization we analyzed fecal samples from 3 separate groups of hospital patients collected after different lenghts of time. For practical reasons one group was studied prospectively and two other groups (patients hospitalized for up to 7 days and patients hospitalized for more than 7 days) were compared to one another.

We looked for the emergence of tellurite resistance among Enterobacteriaceae *using a selective medium, MacConkey potassium tellurite (MCPT). The frequence of prospectively studied patients with tellurite resistant strains was significantly greater after 7 days of hospitalization. For the two other groups, patients with more than 7 days of hospitalization showed a significant increase of bacterial species and of strains with new antimicrobial resistance markers. High molecular weight plasmids were detected in some of these strains.*

These data show that the MCPT medium is a useful tool for the investigation of bowel colonization in hospitalized patients by drug-resistant Enterobacteriaceae.

Key words: selective medium – potassium tellurite – bowel colonization – antimicrobial resistance

Nosocomial infections are known to be related to several factors, as: patients have a decrease in their immunity, due to use of drugs or to underlying diseases; invasive procedures such as surgical operations; defects in the general aseptic procedure; misuse of antimicrobials, leading to selection of resistant microorganisms (Schimpff et al., 1972; Bennett & Brachman, 1979; Dixon, 1981; Cohen et al., 1983). The frequent change in the normal patients microbiota and the resulting colonization by microorganisms more commonly related to nosocomial environment, also contribute to the increase of hospital infections (Montgomerie et al., 1970; Shooter, 1971; Dixon, 1981).

Among bacteria commonly found in association with hospital infections, gram-negative bacilii, specially *Escherichia coli* and *Klebsiella pneumoniae* can be emphasized. Many of them are members of the normal microbiota of the human bowel despite their ability to cause extra-intestinal infections. Thus, intestinal microbiota can be characterized as an important reservoir of bacteria that are frequently isolated from hospital infections (Bennett & Brachman, 1979; Pereira & Suassuna, 1986).

These bacteria frequently show a multiple resistance to antimicrobials related to possession of plasmids, transferable extrachromossomial elements. Plasmids can contain genes that lead to resistance to antibiotics, chemotherapics and certain methalic ions, as mercuric ion and potassium tellurite (Summers & Jacoby, 1977; Pereira, 1990).

Enterobacteriaceae isolated from hospital-

Research supported in part by FINEP (4.3.85.0310.00).
+Research fellow CNPq (402778/84 and 402409/86.8/BM/FV).

Received 27 July 1992.
Accepted 22 December 1992.

acquired infections show a high frequence rate of resistance to tellurite ion. Therefore, it could be used as a marker for strains of *Enterobacteriaceae* related to hospital infections (Pereira, 1982), and lead us to use MacConkey agar with potassium tellurite (MCPT medium) as a useful tool in the investigation of intestinal colonization by *Enterobacteriaceae.*

In this work, we intend to follow the establishment of an antimicrobial resistant microbiota in the intestines of hospitalized patients using MCPT medium. We chose to study broad spectrum antimicrobials as those are widely used in hospital and community prescriptions.

MATERIALS AND METHODS

Patients – 57 patients showing diverse patolhogies, 41 adults and 16 newborns, were randomly sampled from clinical, surgical and maternity wards, in 1986 at the Pedro Ernesto University Hospital _ UERJ (HUPE), a general 600-bed tertiary care hospital. We obtained from medical records data about occurrence and duration of previous hospitalization in HUPE or in another hospital, basic pathology, and day of hospitalization and antimicrobials use.

From 7 patients (patients with two samples, PTS) we were able to obtain two fecal samples, the first one at up to 48 hr of hospitalization and the second one, after 7 days of hospitalization. From 50 patients we were able to obtain only one fecal sample (patients with one sample, POS): from 24 patients we obtained one fecal sample at up to 7 days of hospitalization, and from 26 patients a fecal sample was obtained after 7 days of hospitalization. For 4 POS hospitalized for up to 7 days at HUPE we were not able to obtain data about recent hospitalization in another hospital. The difference of antimicrobial use, between POS hospitalized for up to 7 days and those POS with more than 7 days of hospitalization, was not statistically significant.

Media and culture methods – We used Agar MacConkey (Difco) containing 25 μg/ml of a aqueous solution of potassium tellurite (Merk) (MacConkey-potassium tellurite medium, MCPT medium). Faeces were carefully homogenized in NaCl (0.85%) and diluted 1/20, 1/1000, 1/2000 and 1/5000. Afterwards, 0,1 ml of each dilution were plated on the selective medium. The incubation was performed at 37 °C for 48 hr. The fecal samples showing no growth were assumed as negatives.

Bacterial identification – Identification of *Enterobacteriaceae* was based on colonial morphology, gram stain and biochemical tests, according to Edwards & Ewing (1972). They were also submitted to susceptibility test to tellurite (Pereira & Suassuna, 1986) and to the chosen antimicrobials (ampicillin, amikacin, cephalotin, tetracycline, gentamicin, trimethoprim-sulfamethoxazole, kanamycin and chloranphenicol), according to Bauer et al. (1966). Two strains of the same species were assumed as different when they showed distinct resistance patterns. Strains were assumed as multiresistant when they showed a greater number of resistance markers than the analyzed strains median.

Statistics – The data were analyzed by the chi-square test and by the Fisher's exact test (Berquó et al., 1980).

Plasmids – Plasmid DNA was obtained according to Kado & Liu (1981) and analyzed by agarose gel electrophoresis (0.8% gel) in 40 mM TRIS-2mM EDTA-pH 7.9. The voltage was determined in terms of the molecular weights of the plasmids in analysis (often, 10 V/cm). Agarose gels were stained with ethidium bromide and photographed by transilumination (long UV-300 nm, Brunck & Simpson, 1977) employing red filters and 400 ASA film, according to Kado & Liu (1981) and Maniatis et al. (1982).

RESULTS

We isolated 17 different tellurite resistant strains (TRS) of *Enterobacteriaceae* among PTS, 4 of these strains were obtained from 3 patients at up to 48 hr of hospitalization and 13 of these strains obtained from 7 patients after 7 days of hospitalization. We found a significant increase in the number of patients showing TRS after 7 days of hospitalization ($p = 0.035$) (Table I).

In relation to the patients with one sample (POS), 51 different TRS of *Enterobacteriaceae* were isolated. Fifteen strains were isolated from 11 among 24 patients at up to 7 days of hospitalization (45.8% of these patients) and 36 strains were isolated from 19 among 26 patients hospitalized for more than 7 days (73.1% of these patients) ($p > 0.05$) (Table II). We only obtained complete resistance patterns for 36 strains of the 51 TRS isolated from POS. Among these strains, 80.6% (29/36) were resistant to at least one of the studied antimicrobials (Table III).

TABLE I

Species and antibiotypes of tellurite resistant *Enterobacteriaceae* isolated from hospitalized patients (HUPE-UERJ), in two periods of hospitalization: at up to 48 hr of hospitalization (1st period) and with more than 7 days of hospitalization (2nd period)

Patient	1st period		2 nd period	
	Species[a]	Antibiotype[b]	Species	Antibiotype
TA	Kp	TcAp	Ec	TcApTSKm
			Kp	TcAp
			Kp	Tc
MA	Eag	Tc	Kp	TcApTS
	Kp	TcApTSGm	Kp	TcTS
			Kp	TcAP
WA	Kp	TcApTS	Kp	TcAp
SA	none		Eae	TcApCmTSKmGmCt
IR	none		Kp	TcTS
			Eag	TC
JO	none		Ec[c]	
PC	none		Kp	TcAp
			Ecl	TcApCt

a: Kp = *Klebsiella pneumoniae;* Eag = *Enterobacter agglomerans;* Ec = *Escherichia coli;* Eae = *Enterobacter aerogenes;* Ecl = *Enterobacter cloacae.*
b: Tc = tetracycline; Ap = ampicillin; Cm = chloramphenicol; TS = trimethoprim-sulfamethoxazole; Km = Kanamycin; Gm = gentamicin; Ct = cephalotine.
c: antibiotype not obtained.

TABLE II

Distribution of the fecal tellurite-resistant strains of *Enterobacteriaceae* by species. Strains isolated from 30 among 50 patients hospitalized (HUPE-UERJ): 11 among 24 patients at up to 7 days of hospitalization (1st period) and 19 among 26 patients with more than 7 days of hospitalization (2nd period)

Enterobacteriaceae species	1st period number (%)	2nd period number (%)	Total number (%)
Klebsiella pneumoniae	5 (33,3)	13 (36,1)	18 (35,3)
Escherichia coli	10 (66,3)	5 (13,9)	15 (29,4)
Enterobacter cloacae	0	6 (16,7)	6 (11,7)
Enterobacter sp	0	1 (2,8)	1 (2,0)
Citrobacter freundi	0	3 (8,3)	3 (5,9)
Proteus mirabilis	0	3 (8,3)	3 (5,9)
Proteus vulgaris	0	4 (11,1)	4 (7,8)
Proteus sp	0	1 (2,8)	1 (2,0)
Total	15 (100)	36 (100)	51 (100)

The median number of resistance markers was 2. Resistance to 3 or more markers (drug multiresistance) was not associated to tellurite resistance, but 36.1% (13/36) of analyzed strains showed drug multiresistance (Table III). In relation to drug multiresistance, there were no statistically significant differences comparing strains isolated from patients at up to 7 days of hospitalization and strains isolated from patients hospitalized for more than 7 days (Table III). The statistical analysis of the difference in the numbers of patients with

TABLE III

Distribution of 36 tellurite-resistant strains of *Enterobacteriaceae* by numbers of antimicrobial resistance markers. Strains isolated from 6 patients hospitalized for up to 7 days (1st period) and 14 patients hospitalized for more than 7 days (2nd period), in HUPE-UERJ

Number of resistance markers[a]	Number of strains		
	1st period	2nd period	Total
0	2	5	7
1	3	3	6
2	1	9	10
3	0	5	5
4	1	2	3
5	1	0	1
6	0	1	1
7	0	3	3
Total	8	28	36

a: analyzed antimicrobials: tetracycline; ampicillin; cloramphenicol; trimethoprim-sulfametoxazole; kanamycin; amikacin; cephalotion.

multiresistant strains when comparing patients hospitalized at up to 7 days and patients hospitalized for more than 7 days was not possible because some of the strains were not tested, as to their susceptibility to antimicrobials. However, there were greater numbers of multiresistant strains in patients with more than 7 days of hospitalization (Table III) and new markers for antimicrobial resistance (as for example, gentamicin, amikacin and cephalotin) appeared among the tellurite resistant strains isolated from POS after 7 days of hospitalization. The frequence of those markers, taken alone or in association, was significantly greater for POS with more than 7 days of hospitalization ($p = 0.035$).

The number of different strains was greater for PTS and POS hospitalized for more than 7 days (Table I, IV).

TABLE IV

Enterobacteriaceae species of fecal tellurite-resistant strains isolated from 30 among 50 patients hospitalized (HUPE-UERJ): 11 among 24 patients at up to 7 days of hospitalization (1st period) and 19 among 26 patients with more than 7 days of hospitalization (2nd period)

Species of *Enterobacteriaceae*	Patients[a] 1st period number (%)	2nd period number (%)
Klebsiella pneumoniae	5 (20,8)	11 (42,3)
Escherichia coli	8 (33,3)	4 (15,4)
Enterobacter cloacae	0	3 (11,5)
Enterobacter sp	0	1 (3,8)
Citrobacter freundi	0	2 (7,7)
Proteus mirabilis	0	3 (11,5)
Proteus vulgaris	0	4 (15,4)
Proteus sp	0	1 (3,8)
None	13 (54,2)	7 (26,9)

a: one patient may show more than one *Enterobacteriaceae* species of tellurite-resistant st in.

Among the PTS group, t' e bacterial species of TRS isolated at up to 7 days of hospitalization were *K. pneumoniae* and *E. coli* (with one exception) (Table I). A greater variety of bacterial species was isolated from patients hospitalized for longer periods. Presence of species other than *K. pneumoniae* and *E. coli* was significantly greater in patients with more than 7 days of hospitalization (Table IV). These species correspond to those more prevalent in hospital infections than in community infections, according to Bennett & Brachman, 1979, and Dixon, 1981. The frequence of these species was significantly greater in POS with more than 7 days of hospitalization ($P < 0.001$).

Almost all analyzed *Enterobacteriaceae* isolated in MCTP medium have shown the presence of high molecular weight plasmids (Figs 1, 2).

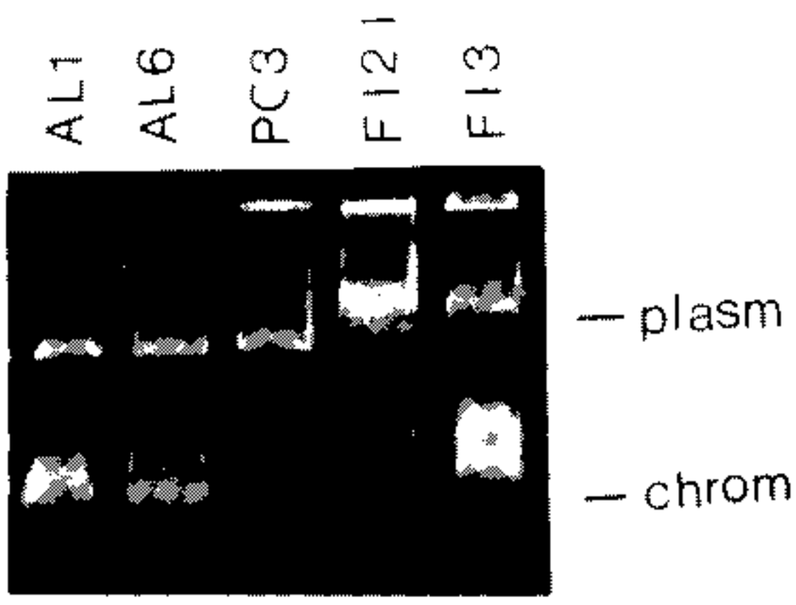

Fig. 1: agarose gel electrophoresis of plasmids found in strains of *Enterobacter cloacae* isolated from faeces from patients hospitalized for up to 7 days (1st period) and for more than 7 days (2nd period). AL 1 – Tc Te; AL 6 Ak Ap Te; from 2nd period and PC 3 – Ap Cm Tc Te; FI 2 Ap Cm Tc; FI 3 – Ap Cm Tc Tp Te TS from 1st period.
Resistance markers: Ap = ampicillin; Ak = amikacin; Cm = chloramphenicol; Gm = gentamicin; Km kanamycin; Tc = tetracycline; Te = tellurite; TS = trimethoprim-sulfamethoxazole.

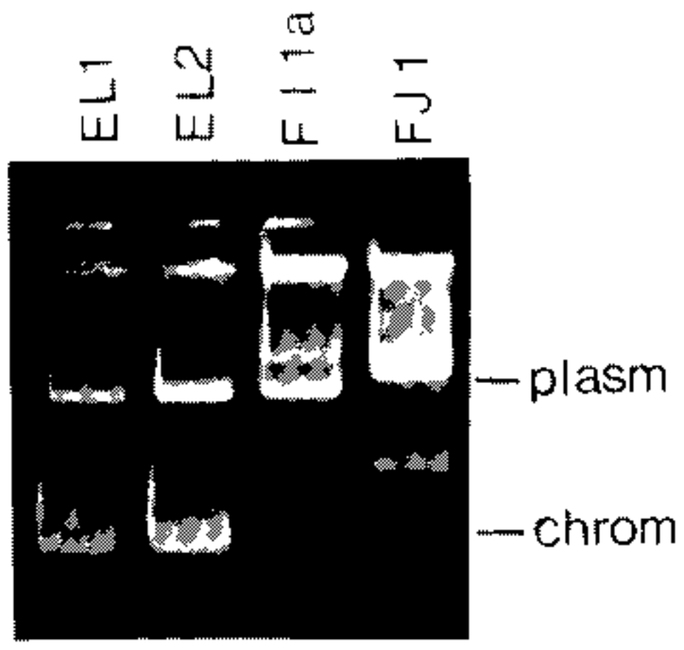

Fig. 2: agarose gel eletrophoresis of plasmids found in strains of *Klebsiella pneumoniae* isolated from faeces from patients hospitalized for up to 7 days (1st period) and for more than 7 days (2nd period). EL 1 - Tc Te TS; EL 2 – Tc Te TS; FJ1 – Tc Te, from 1st period and FI1a – Ap Gm Km Tc Te, from 2nd period.
Resistance markers: Ap = ampicillin; Ak = Amikacin; Cm = chloramphenical; Gm = gentamicin; Km = kanamycin; Tc = tetracycline; Te = tellurite; TS = trimethoprim-sulfamethoxazole.

DISCUSSION

There were no statistically significant differences in the frequence of the tellurite resistant strains (TRS) when comparing patients with one sample (POS) at up to 7 days and those tested at more than 7 days of hospitalization in contrast to the patients with two samples (PTS). This fact probably occurred because the prospective approach of the PTS group would more adequately manifest the differences attributable to nonocomial colonization such as intestinal colonization by TRS. It is also important to note the greater time interval between the two periods in the PTS group. However, the MacConkey-potassium tellurite (MCPT) medium was able to detect differences between TRS isolated from patients hospitalized for up to 7 days and those isolated from patients after 7 days of hospitalization.

In spite of eventual differences in the intestinal colonization of adults and newborns, the heterogeneity of the analyzed sample did not seem to interfere with our findings, as the numbers of adults and newborns were almost identical among POS hospitalized for up to 7 days (8 newborns and 16 adults) and for more than 7 days (8 newborns and 18 adults).

We have verified that the differences in the frequence of tellurite resistance between the first and second periods was not significant when adults were analyzed; among newborns, we have found a marginally non-significant value ($p = 0,056$). Due to the small number of newborns studied, a definite conclusion could not be reached. This point will be taken up again in a further work.

The choice of MacConkey-potassium tellurite medium is based on the plasmidial codification of tellurite resistance (Summers & Jacoby, 1977) and because of its frequent association to antimicrobials resistance (Pereira & Suassuna, 1986). Therefore, the use of selective media for TRS may detect nosocomial microorganisms. These microorganisms usually show greater numbers of resistance markers, frequently codified by plasmids. We have previously studied cases of nosocomial infections caused by tellurite-resistant Klebsiella pneumoniae, and we have detected single transferable high molecular plasmids, codifying both tellurite resistance and antimicrobial bacterial resistance (Pereira, 1990).

Multiresistance was not associated to tellurite resistance, despite previous data for *Enterobacteriaceae* strains isolated from nosocomial infections. This probably occurs bacause tellurite resistance is plasmidial and among bacterial strains causing nosocomial infections, plasmids frequently carry multiple drug-resistance. However, this phenomenon does not necessarity occur in microbiota bacterial strains. On the other hand, we found a greater number of multiresistant strains among patients hospitalized for more than 7 days. A definitive statistical analysis was not possible because some data related to antimocribials resistance were not obtained.

Bacterial strains isolated from patients after 7 days of hospitalization showed a greater frequence of markers for gentamicin and/or amikacin and/or caphalotin. These antimicrobials actually determine a greater selective pressure due to their extensive use in the nosocomial environment (Kunin, 1985; Pancoast, 1988).

In a previous study (Pereira, 1990), we verified that in community-acquired urinary infections (in women), there is a tendency to independence of association to antimicrobial resistance markers in the ethiologic agents; these findings contrast with the dependence noted among resistance markers in nosocomial urinary infections, mainly related to strains showing resistance to gentamicin and/or amikacin and/or cephalotin.

The frequent detection of high molecular weight plasmids in the tellurite resistant strains confirmes our previous work (Pereira, 1990). We did not analyze whether they are able to transfer resistance by conjugation; this fact prevents us from being able to affirm that the tellurite resistance is codified by those plasmids. However, in a previous study with a greater number of *K. pneumoniae* strains, it was shown that a single high molecular weight plasmid codifies both transferable tellurite resistance and antimicrobial resistance (data not published).

It was technically difficult to detect those plasmids because they occur in a low number of copies in each cell and because of the difficulty in separating them due to their size from other cellular components during the extractive step (Griffin et al., 1985). Anyway, it is necessary to extract plasmid DNA from a

great number of cells, leading to problems in relation to plasmid purification (Hardy, 1987; Couturier et al., 1988).

Tomás et al. (1986) described an analogous medium for detection of environmental strains, but employing lower concentrations of potassium tellurite than those used by us. From our point of view, that medium is inadequate for the study of hospital strains resistance, because the prevalence of tellurite resistant *Enterobacteriaceae*, with plasmid codification, is extremely high. Besides, the codification gives rise to resistance levels (Mic = 25 μg/ml) that contrast with data from their study (3 μg/ml).

We believe that our selective medium would be useful to trace plasmid-mediated tellurite resistance in hospitals and in other environments.

ACKNOWLEDGEMENTS

To Dr João Ramos Costa Andrade for critical suggestions; to Dr Adriano Caldeira de Araújo for colaboration on the statistical analysis and to Edson Nóbrega da Silva for secretarial work.

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