Variants of *Escherichia coli* giving the appearance of mixed growths in urine

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**SYNOPSIS** Urines from patients with symptoms of urinary tract infection yielded mixed growths of different colony types of *Escherichia coli*. The different colony types were found to be variants of single infecting strains caused by mutation or by phage action. It is suggested that care should be exercised in the interpretation of apparently mixed growths from urine.

A mixed growth from a mid-stream specimen of urine is generally considered to indicate contamination during collection of the urine or, very rarely, a mixed infection. This paper describes three cases in which strict application of this generalization might have led to a misleading report.

**Case 1**

A specimen of mid-stream urine from a woman with frequency of micturition, dysuria, and back pain was examined routinely for cells and culture. The cell count in a modified Fuchs Rosenthal chamber showed leucocytes >1000 per µl and red blood cells <1 per µl. The urine was cultured by a standard (3 mm) wire-loop method on Oxoid Cystein Lactose Electrolyte Deficient Agar (CLED).

After incubation at 37°C for 18 hours there was an apparently mixed growth of three organisms, each producing a different type of colony and presenting between 10 000 and 100 000, with a total of more than 100 000 organisms per ml of urine (fig 1, which is not, however, the original plate). The three colony types were all 2 mm in diameter, circular, convex, and entire. One was an irregular surfaced bright lactose fermenter; the second was an irregular surfaced non-lactose fermenter which on re-incubation for 48 hours was weakly lactose-fermenting; the third was a smooth-surfaced lactose fermenter. All three organisms retained their distinctive colony types when subcultured on CLED, MacConkey’s, and blood agar. When kept at room temperature for two weeks a fine surface growth appeared around all three colony types, and on subculture this growth yielded identical colony forms with the exception of the smooth lactose fermenter which yielded a pure growth of bright lactose-fermenting colonies.

All three organisms gave identical biochemical reactions after 24 and 48 hours and after incubation for five days at 37°C. They also gave similar inhibition-zone diameters (maximum difference 2 mm) to a range of antibacterial drugs; when compared with a sensitive control (*E. coli* NCTC no. 10418), they were sensitive to ampicillin, sulphafurazole,

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Received for publication 4 March 1975.
co-trimoxazole, trimethoprim, nitrofurantoin, cepha-
lexin, carbenicillin, gentamicin, polymyxin, nal-
adixic acid, kanamycin, neomycin, streptomycin,
and chloramphenicol, and resistant to tetracycline.

The three organisms were serotyped and all were
found to be *E. coli* 0101. The smooth lactose
fermenter, but not the other two strains, also
possessed the K30 (A) capsular antigen. Phage could
not be demonstrated after filtration of broth cultures
of the three organisms. It is thought that the parent
strain was the one that possessed the K30 (A)
antigen.

*E. coli* 0101 is not one of the most common O
serotypes found in urinary tract infections (Spencer
*et al*, 1968; O'Grady *et al*, 1970; Mabeck *et al*, 1971;
Dootson *et al*, 1973) although it has been isolated
from this source (Grüneberg *et al*, 1968). From
consideration of the clinical history and laboratory
tests it is suggested that the patient was suffering
from a urinary tract infection caused by *E. coli* 0101.

**Case 2**

A mid-stream specimen of urine from a male patient
with dysuria, pyrexia, and loin pain showed a cell
count of leucocytes > 1000 and red blood cells < 1
per μl.

After incubation at 37°C for 18 hours there was
an apparently mixed growth of three organisms,
each representing more than 100 000 organisms per
ml of urine. One colony type was 0-5 mm irregular
surfaced, bright lactose fermenter; the second was
0-5 mm irregular surfaced, pale lactose fermenter;
the third was a minute colony. Only the pale lactose
fermenter grew pure on subculture; the other two
colonies produced growths similar to that of the
original plate.

Single isolated colonies of the three colony types
were grown in broth and were used for biochemical
tests. All three types gave identical biochemical
reactions. They also gave similar inhibition-zone
diameters to a range of antibacterial drugs; when
compared with a sensitive control they were sen-
sitive to ampicillin, sulphafurazole, co-trimoxazole,
tetracycline, nitrofurantoin, cephalaxin, carbenicillin,
gentamicin, polymyxin, naladixic acid, kanamycin,
and streptomycin.

CLED agar plates were flooded with broth culture
of the three colony types; the broths were then
filtered, checked for sterility, and titrated on the
three inoculated plates for the presence of phage
(fig 2). Filtrate from the pale lactose fermenter did
not contain phage whereas the other two did. There
was deepening of the yellow indicator around the
plaques. The bacteria-free filtrate from the bright
lactose fermenter was then added to broth containing
the pale lactose fermenter, and this was then
inoculated onto a fresh CLED plate. After 18 hours'
incubation at 37°C the three colony types were
present.

From these results it was concluded that only one
bacterial strain was present and that this was acted
on by a phage to give colonial variants. The increased
brightness of the affected colonies may be due to
phage lysing the bacterial cell wall, releasing the
galactosidase normally held inside. The organism
was serotyped and was found to be *E. coli* 02, which
is common in urinary tract infections (Grüneberg
*et al*, 1968; Spencer *et al*, 1968; O'Grady *et al*, 1970;
Mabeck *et al*, 1971; Dootson *et al*, 1973). From
consideration of the clinical history and laboratory
tests it is suggested that the patient was suffering
from a urinary tract infection caused by *E. coli* 02.

**Case 3**

A mid-stream specimen of urine from a female
patient with peripheral vascular disease, uraemia,
and anaemia showed a cell count of leucocytes 110
and red blood cells < 1 per ml. After incubation at
37°C for 18 hours there was an apparently mixed
growth of two organisms, each representing
> 100 000 organisms per ml of urine. One colony
was 1 mm irregular surfaced, bright lactose fer-
menter and the other 1·5 mm irregular surfaced,
pale lactose fermenter. Both types grew pure on subculture.

Both colony types gave identical biochemical reactions and similar zone inhibition diameters to a range of antibacterial drugs; when compared with a sensitive control they were sensitive to ampicillin, sulphonamide, co-trimoxazole, tetracycline, nitrofurantoin, and cephalaxin.

Both colonies were serotyped and found to be E. coli 075, which is common in urinary tract infections. From consideration of the clinical history and laboratory tests it is suggested that the patient was suffering from a urinary tract infection caused by E. coli 075.

Discussion

A mixed growth from a mid-stream specimen of urine is generally considered to have been derived from contamination during collection. The appearance of several distinct colony types in moderate numbers from a specimen might prompt the reporting of ‘a mixed growth of doubtful significance’. It is suggested that care be exercised in the reporting of mixed growths and that the findings can be checked by culture of more specimens.

I am grateful to Dr B. Rowe, of the Salmonella and Shigella Reference Laboratory, Colindale for typing the organisms, and to Mrs J. Turner for secretarial help.

References


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*J Clin Pathol* 1975 28: 728-730
doi: 10.1136/jcp.28.9.728

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