

## Relationship between susceptibility to antimicrobials and virulence factors in *Escherichia coli* isolated from food in Morocco

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**Abstract:** The in vitro susceptibilities to several antibiotics of 140 *Escherichia coli* (*E. coli*) isolated from food were analysed. Eighty five (61%) of the isolated *E. coli* strains did not contain virulence factors, whereas 55 (39%) of them contained one or more virulence factors. *E. coli* strains were analysed by polymerase chain reaction for genes encoding the following virulence factors: aerobactin (*iucD*); EAaggEC heat-stable enterotoxin (*astA*); intimin (*eaeA*); shigatoxin (*vt*); pyelonephritis-associated pili (*pap*). It was observed that the virulence genes increased antibiotic resistance of resistant strains and

### Introduction

The dissemination of antibiotic resistance genes among bacterial strains is an increasing problem in infectious diseases. The use of antimicrobials in any venue, including growth promotion in veterinary medicine, can potentially lead to widespread dissemination of antimicrobial-resistant bacteria. In the case of *E. coli*, resistance to tetracyclines, sulfonamides, and streptomycin or spectinomycin is generally the most prevalent (<http://www.arsu.saa.ars.usda.gov/>, ; Guerra *et al.*, 2003; Lanz *et al.*, 2003; Anonymous, 2004; DANMAP, 2004). Antimicrobial resistance is also typically more frequent among pathogens than among commensal bacteria (DANMAP, 2004). This difference has generally been attributed to the more intense and repeated exposure of pathogens to antimicrobial agents. However, to the best of our knowledge, this hypothesis has never been formally tested and other factors may be at work. Physical linkages between antimicrobial resistance genes and specific virulence genes in pathogens may be another explanation (Martinez *et al.*, 2002). Such linkages of genes on large transferable plasmids have been described sporadically in the past for enterotoxigenic *E. coli* (ETEC) isolates from swine and calves (Mazaitis *et al.*, 1981; Franklin & Mollby, 1983; Harnett & Gyles, 1985; Guerra *et al.*, 2003) and for avian *E. coli* isolates (Riley *et al.*, 1983). Nothing is known about the frequency of these associations among field isolates.

The first objective of this study was to obtain an estimate of frequency of resistance to common antimicrobial agents in *E. coli* isolates obtained from food in Casablanca by an internationally standardized method. The second objective was to assess the

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distribution of the major virulence genes in these isolates and identify any associations between virulence and resistance in *E. coli* isolates.

### Materials and methods

**Bacterial strains.** A total of 140 *E. coli* strains recovered from between March 2004 and July 2006 in Casablanca, Morocco were examined. The strains were isolated mainly from ground beef, turkey and sausage. *E. coli* identification was done by biochemical methods and, for confirmation, the  $\beta$ -*Glu* gene of *E. coli* was amplified by PCR (Bej *et al.*, 1991).

**Extraction of bacterial DNA and PCR.** Bacterial DNA was extracted by Instagene Matrix kit (Biorad) as described by the manufacturer. The *E. coli* DNA was tested for the presence of 5 virulence genes associated with strains causing intestinal and extra-intestinal infections. The virulence genes, included *stx*, *eaeA*, *iucD*, *astA*, and *pap*, are those used by (Badri *et al.*, 2009).

**Antimicrobial susceptibility testing.** The antimicrobial susceptibilities of *E. coli* isolates were determined by the disk diffusion method of the Clinical and Laboratory Standards Institute on Mueller–Hinton agar (Biorad). The 16 antibiotics tested were the following: (a) those commonly used in veterinary medicine, including amoxicilline (AMX), amoxicillin+clavulanic acid (AMC), ticarcilline (TIC), tetracycline (TE), streptomycin (S), gentamicin (GM), kanamycin (K), and chloramphenicol (C) (although not authorized for use in food animals); and (b) antibiotics employed mainly in humans: cephalothin (CF)\*, cefotaxime (CTX), norfloxacin (NOR), ciprofloxacin (CIP)\*, cefoxitin (FOX), amikacin (AN), nalidixic acid (NA) (\*used in some animals). Cotrimoxazole (SXT) was also included (trimethoprim combined with sulfamethoxazole).

**Statistical analysis.** Statistical analyses were performed using  $\chi^2$  and Fisher's exact tests (Dowson-Sounders & Tropp, 1990).

### Results

This study focused on 140 *E. coli* strains isolated from food. The in vitro susceptibilities to various antibiotics of *E. coli* strains containing *iucD*, *astA*, *eae*, *vt* and *pap* virulence factors analysed by PCR were studied. Eighty five (61%) of the isolated *E. coli* strains did not contain virulence factors, whereas 55 (39%) of them contained one or more virulence factors. In the total population, the number of isolates containing each virulence factor was as follows: 20(14%) *iucD*; 11(8%) *astA*; 8(6%) *eae*; 2(1%) *vt*; 2(1%) *pap*; 9 (6%) *astA* + *iucD*; 1(1%) *astA* + *pap*; 1(1%) *iucD* + *pap*; 1(1%) *eae* + *astA* + *pap* (Table 1).

When resistance to AMC, CF, FOX, and AN of *E. coli* strains that did not contain virulence genes was compared with strains that contained different virulence genes, it was observed that the resistance was disappeared in all isolates that contained virulence genes. There was no resistance to CTX was observed in all strains tested except one strain contained *eae* gene.

When resistance of *E. coli* strains that did not contain virulence genes was compared with those that contained at least one virulence factor, resistance to GM was disappeared in all isolates that contained virulence genes except one strain with *astA* + *iucD* genes combination.

Comparing strains with *astA* + *iucD* with those with no virulence factors, resistance to TE, C, STX, NA, NOR, CIP, S and K increased. There was no difference in resistance to TIC between isolates that did not contain virulence genes with those that contained at least one virulence factor. The resistance to AMC, C, SXT, NA, NOR, S and K was increased in strains having *astA* + *iucD* but in strains harbouring only *iucD* resistance decreased (the difference was statistically significant ( $P < 0,001$ )).

### Discussion

In *E. coli* strains containing certain gene groups, the increase in resistance was between 24% and 74% for AMX, between 12% and 79% for TIC, between 1% and 41% for TE, between 4% and 51% for C, between 38% and 88% for SXT, between 37% and 87% for NA and NOR, between 2% and 93% for CIP, between 24% and 74% for S and between 27% and 94% for K. The results of our study are supported by a study by Orden *et al.* indicating that *E. coli* virulence factors could be the reason for resistance to different antibiotics (Orden *et al.*, 2000). In their study, Katouli *et al.* found that 53% of *E. coli* isolates showed high resistance to ampicillin and TMP/SMX antibiotics.

In another study by Vranes *et al.*, *E. coli* strains harbouring the *pap* gene showed resistance to amoxicillin (Vranes *et al.*, 1994). In our study, *E. coli* strains containing the *pap* gene were also more resistant to AMX, TIC, TE, SXT, NA, NOR, CIP, S and K compared with those not containing the *pap* gene.

Maynard *et al.* studied the relationships between virulence factors and antimicrobial resistance and found that *E. coli* strains frequently showed resistance to ampicillin, tetracycline and sulfonamides (Maynard *et al.*, 2004).

In our study, the gene combination *astA*+*iucD* increased resistance to AMX, whilst for other gene combinations 100% sensitivity to this antibiotic was noted. A similar situation was also observed with TIC, C, NA, NOR, CIP, S and K, where with most gene combinations nearly 100% sensitivity was observed against these antibiotics. These results show that *E. coli* strains with certain virulence factors compared with

those not having them were sensitive to the same antibiotic groups, as in the study of Orden et al. (Orden et al., 2000).

In our study, when we evaluated the antibiograms of *E. coli* strains having virulence genes and those not having virulence genes, we found that virulence genes increased the resistance in resistant strains and increased sensitivity in susceptible strains.

It can be concluded that emergence and dissemination of antimicrobial resistance in *E. coli* strains containing virulence factors may complicate treatment of certain urinary tract and enteric infections in humans and animals. The data did suggest that antimicrobial use in agriculture was important in the selection of antimicrobial-resistant phenotypes. Continued surveillance of *E. coli* collected from agricultural and clinical settings, including the food production, is merited to identify emerging antimicrobial-resistant phenotypes.

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Table 1: Effect of virulence genes on resistance of 140 *E. coli* isolates to various antibiotics  
 NVF, no virulence factors.

| Gene                | No.     | No. (%)<br>AMX | No. (%)<br>AMC | No. (%)<br>TIC | No. (%)<br>TE | No. (%)<br>CF | No. (%)<br>FOX | No. (%)<br>CTX | No. (%)<br>AN | No. (%)<br>GM | No. (%)<br>C | No. (%)<br>SXT | No. (%)<br>NA | No. (%)<br>NOR | No. (%)<br>CIP | No. (%)<br>S | No. (%)<br>K |
|---------------------|---------|----------------|----------------|----------------|---------------|---------------|----------------|----------------|---------------|---------------|--------------|----------------|---------------|----------------|----------------|--------------|--------------|
| <b>NVF</b>          | 85 (61) | 22 (26)        | 7 (8)          | 18 (21)        | 50 (59)       | 8 (9)         | 6 (7)          | 0              | 1 (1)         | 3 (4)         | 4 (5)        | 10 (12)        | 11 (13)       | 11 (13)        | 6 (7)          | 22 (26)      | 5 (6)        |
| <i>iucD</i>         | 20 (14) | 1 (5)          | 0              | 2 (10)         | 12 (60)       | 0             | 0              | 0              | 0             | 0             | 1 (5)        | 1 (5)          | 2 (10)        | 1 (5)          | 3 (15)         | 3 (15)       | 1 (5)        |
| <i>astA</i>         | 11 (8)  | 0              | 0              | 0              | 9 (82)        | 0             | 0              | 0              | 0             | 0             | 1 (9)        | 1 (9)          | 1 (9)         | 1 (9)          | 1 (9)          | 1 (9)        | 0            |
| <i>eae</i>          | 8 (6)   | 0              | 0              | 1 (13)         | 5 (63)        | 0             | 0              | 1 (13)         | 0             | 0             | 0            | 0              | 0             | 0              | 0              | 2 (25)       | 0            |
| <i>vt</i>           | 2 (1)   | 0              | 0              | 0              | 1 (50)        | 0             | 0              | 0              | 0             | 0             | 0            | 0              | 0             | 0              | 0              | 0            | 0            |
| <i>pap</i>          | 2 (1)   | 1 (50)         | 0              | 1 (50)         | 1 (50)        | 0             | 0              | 0              | 0             | 0             | 0            | 1 (50)         | 1 (50)        | 1 (50)         | 1 (50)         | 1 (50)       | 1 (50)       |
| <i>astA+iucD</i>    | 9 (6)   | 5 (56)         | 0              | 3 (33)         | 9 (100)       | 0             | 0              | 0              | 0             | 1 (11)        | 5 (56)       | 7 (78)         | 8 (89)        | 6 (67)         | 5 (56)         | 8 (89)       | 3 (33)       |
| <i>astA+pap</i>     | 1 (1)   | 1 (100)        | 0              | 1 (100)        | 1 (100)       | 0             | 0              | 0              | 0             | 0             | 0            | 1 (100)        | 1 (100)       | 1 (100)        | 1 (100)        | 1 (100)      | 1 (100)      |
| <i>iucD+pap</i>     | 1 (1)   | 1 (100)        | 0              | 1 (100)        | 1 (100)       | 0             | 0              | 0              | 0             | 0             | 0            | 1 (100)        | 0             | 0              | 0              | 0            | 0            |
| <i>eae+astA+pap</i> | 1 (1)   | 0              | 0              | 0              | 1 (100)       | 0             | 0              | 0              | 0             | 0             | 0            | 1 (100)        | 1 (100)       | 0              | 0              | 1 (100)      | 1 (100)      |