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## **Integrans, a predictive biomarker for antibiotic resistance in acute sepsis: the IRIS study**

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HAL Authorization

1 **Integrans, a predictive biomarker for antibiotic resistance in acute sepsis.**

2 **The IRIS study.**

3

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24

25 **Running title:** Integrans as predictive markers of AMR

## 26 **Synopsis**

27 **Objectives:** Considering the increase in MDR Gram-negative bacteria (GNB), the choice of  
28 empirical antibiotic therapy is challenging. In parallel, use of broad-spectrum antibiotics  
29 should be avoided to decrease antibiotic selection pressure. Accordingly, clinicians need rapid  
30 diagnostic tools to narrow antibiotic therapy. Class 1-3 integrons, identified by *intI1-3* genes,  
31 are genetic elements that play a major role in antibiotic resistance in GNB. The objective of  
32 the IRIS study was to evaluate the negative and positive predictive values (NPV-PPV) of  
33 *intI1-3* as markers of antibiotic resistance.

34 **Methods:** The IRIS study is an observational cross-sectional multicentre study that enrolled  
35 adult subjects with suspected urinary tract or intra-abdominal infections. *intI1-3* were detected  
36 directly from routinely collected biological samples (blood, urine or intra-abdominal fluid)  
37 using qPCR. A patient was considered “MDR-positive” if at least one GNB, expressing  
38 acquired resistance to at least two antibiotic families among  $\beta$ -lactams, aminoglycosides,  
39 fluoroquinolones and/or cotrimoxazole, was isolated from at least one biological sample.

40 **Results:** Over a 2-year-period, 513 subjects were enrolled and 409 had GNB documentation,  
41 mostly enterobacterales. *intI1* and/or *intI2* were detected in 31.8% of patients and 24.4% of  
42 patients were considered “MDR-positive”. The NPV of *intI1* and/or *intI2* as a marker of  
43 acquired antibiotic resistances was estimated at 92.8% [89.1-95.5]. The NPVs for first-line  
44 antibiotics were all above 92%, notably >96% for resistance to third generation  
45 cephalosporins.

46 **Conclusions:** The IRIS study strongly suggests that absence of *intI1* and *intI2* from biological  
47 samples in patients with GNB-related infections is predictive of the absence of acquired  
48 resistances.

49

50

## 51 **Introduction**

52       The incidence of sepsis increases, mainly because of population aging and rise of  
53 immunosuppression.<sup>1</sup> Clinicians usually prescribe empiric broad-spectrum antibiotics to get  
54 around the time needed to obtain the susceptibility profile of the causative pathogen, and the  
55 emergence of MDR Gram-negative bacteria (GNB).<sup>2</sup> This issue is even worse in sepsis  
56 caused by intra-abdominal and urinary tract infections (UTI) in which prevalence of GNB is  
57 important. In the era of increasing antimicrobial resistance (AMR), it is fundamental to detect  
58 MDR-GNB. Still, it is also crucial to detect susceptible GNB to preserve broad-spectrum  
59 antibiotics and to decrease selective pressure from unnecessary antibiotic use.

60       Rapid diagnostic tools with easy-to-detect biomarkers would be helpful. Non-culture-  
61 based methods have been developed<sup>3,4</sup> but they provide little information on AMR of GNB  
62 and they often lack sensitivity to be used directly on biological samples. Clinical  
63 metagenomics brought new insights<sup>5,6</sup> but the current long turnaround time and the cost are  
64 not compatible yet with clinical needs. Class 1-3 integrons are genetic elements involved in  
65 AMR among GNB.<sup>7</sup> **Integrons** can promote the acquisition and expression of antibiotic  
66 resistance genes (ARGs) embedded within gene cassettes. Detection of gene cassettes is not  
67 appropriate because hundreds have been described.<sup>8</sup> However, integrons all have an *intI*  
68 integrase gene that can be easily detected. Several studies underlined the link between class 1-  
69 **2** integrons and MDR-GNB<sup>9-11</sup> even if resistances are not due only to cassette-borne genes  
70 but to other ARGs located outside the integrons. More recent studies underlined the fact that  
71 absence of *intI1-3* is associated with the absence of acquired resistances, notably for  
72 resistance to third generation cephalosporins (3GC), aminoglycosides and fluoroquinolones.  
73 <sup>10, 11</sup>

74 The objective of the IRIS study was to evaluate the utility of *int11-3* to predict AMR, by  
75 detecting them directly from biological samples in patients with urinary or intra-abdominal  
76 sepsis.

77

## 78 **Methods**

### 79 *Study population*

80 The IRIS study was a multicentre observational cross-sectional study approved by the  
81 Ethics Committee of Limoges University Hospital (No 99-2012-24) that agreed to a waiver of  
82 consent. Patients were enrolled over a 2-year-period in Emergency Departments (ED) and  
83 ICUs from eight French hospitals when admitted for acute sepsis from urinary or intra-  
84 abdominal origin, as defined by the international consensus conferences.<sup>12, 13</sup> Patients were  
85 excluded if younger than 18 years old, pregnant or breastfeeding.

86

### 87 *Biological samples*

88 One blood sample, blood cultures and at least one biological sample (urine or intra-  
89 abdominal fluid when appropriate) were routinely collected at admission. Culture was  
90 performed as recommended by ESCMID  
91 ([https://www.escmid.org/escmid\\_publications/manual\\_of\\_microbiology/](https://www.escmid.org/escmid_publications/manual_of_microbiology/)). Identification was  
92 performed using MALDI-TOF mass spectrometry. Antimicrobial susceptibility testing was  
93 performed following EUCAST recommendations (<http://www.eucast.org/>). Blood samples  
94 and biological fluids were stored at -80°C.

95

### 96 *Multidrug resistance*

97 Definition of multidrug resistance is not consensual. Here, we chose stringent criteria to  
98 define MDR-GNB. Ten antibiotics commonly used to treat GNB-related UTI<sup>14</sup> or intra-

99 abdominal infections<sup>15</sup> were considered: amoxicillin, amoxicillin and clavulanic acid,  
100 piperacillin and tazobactam, cefotaxime, ceftazidime, imipenem, gentamicin, amikacin,  
101 ciprofloxacin (or ofloxacin) and cotrimoxazole. A patient was considered “MDR-positive” if  
102 at least one MDR-GNB (*i.e.* expressing acquired resistance to at least two antibiotic families  
103 among  $\beta$ -lactams, aminoglycosides, fluoroquinolones and cotrimoxazole) was isolated from at  
104 least one biological sample.

105

#### 106 *Detection of int11-3*

107 All biological samples were sent to Limoges for *int11-3* genes detection. The whole  
108 process took 2 h. DNA was extracted using the NucliSENS® easyMAG™ system from one  
109 millilitre of sample. *int11-3* were detected with a multiplex qPCR.<sup>16, 17</sup> The absence of PCR  
110 inhibitors was checked by amplifying the  $\beta$ 2-microglobulin gene. If at least one of *int11*, *int12*  
111 and/or *int13* was detected from at least one biological sample of one given patient, the patient  
112 was considered “*int11-3*-positive”.

113

#### 114 *Data management and statistical analysis*

115 All data were collected into an electronic case report form. An adjudication committee  
116 with one intensivist, one emergency physician and one microbiologist, blindly reviewed all  
117 cases. Patients with non GNB-related sepsis were secondarily excluded.

118 Negative and positive predictive values (NPV, PPV) of *int11 and/or int12* as a marker of  
119 acquired resistances were estimated, as well as the sensitivity, the specificity, the positive and  
120 the negative likelihood ratio. The NPV/PPV of *int11 and/or int12* was also estimated for each  
121 antibiotic. To take into account correlation in data (sometimes several biological samples for  
122 the same patient), we used 10,000 bootstrap samples to generate 95% CI. SAS version 9.4  
123 (SAS Institute, Cary, NC) and R version 3.3.1 were used for statistical analyses.

124

125

126

## 127 **Results**

128 *Patients flow-chart* (Figure S1)

129 A total of 513 patients were enrolled in the study. One hundred and four patients were  
130 secondarily excluded, mainly for absence of GNB or unconfirmed origin of sepsis. Among  
131 the 409 remaining patients, 343 (83.9%) had a urinary sepsis and 66 (16.1%) intra-abdominal.

132

133 *Microbiological data* (Table S1)

134 Among patients with urinary sepsis, 151 (44.0%) had positive blood cultures. Urines  
135 were positive with one GNB for 303 patients and with 2 GNB for 24 patients. Among patients  
136 with intra-abdominal sepsis, 20 (30.3%) had positive blood cultures. Intra-abdominal fluids  
137 were positive with one GNB in 42 patients and with at least 2 GNB in 24 patients.

138 Enterobacterales represented 95.0% of all GNB, *Escherichia coli* first (73.0%),  
139 followed by *Klebsiella pneumoniae* (6.4%) and *Proteus mirabilis* (5.4%). Regarding *E. coli*,  
140 53% were resistant to amoxicillin, 37% to amoxicillin and clavulanic acid, 4.5% to  
141 cefotaxime, 4.5% to gentamicin, 12% to fluoroquinolones and 22% to cotrimoxazole.  
142 Prevalence of “MDR-positive patients” was 24.2% (99 out of 409 patients).

143

144 *Link between **intI genes** and resistance*

145 Because of PCR inhibitors, the final analysis was performed **for samples from** 406  
146 patients (Table 1). **intI1 and/or intI2** were detected in 129 patients (31.8%): 101 (29.6%) with  
147 urinary sepsis and in 28 (43.1%) with intra-abdominal sepsis. One hundred and one (78.3%)

148 patients were *intI1*-positive, 11 (8.5%) were *intI2*-positive and 17 (13.2%) were both *intI1* and  
149 *intI2*-positive. No *intI3* gene was detected.

150 Sensitivity of the test was 79.8% [70.5-87.2] whereas specificity was 83.7% [79.1-  
151 87.7] (Table S2). Twenty patients negative for *intI1-3*- were infected with MDR-GNB. Most  
152 of these patients had non-severe UTI-related sepsis due to *E. coli* with resistance to narrow-  
153 spectrum  $\beta$ -lactams and fluoroquinolones or cotrimoxazole.

154 The PPV and NPV of *intI1* and/or *intI2* as a marker of MDR were 61.2% [52.3-69.7]  
155 and 92.8% [89.1-95.5] respectively (Tables S2-S3). Simulations according to prevalence of  
156 resistances are presented in Figure 1. The NPVs of *intI1* and/or *intI2* were >90% for all first-  
157 line antibiotics with the highest ( $\geq 97.0\%$ ) for ceftazidime, gentamicin, amikacin and  
158 cotrimoxazole. Overall, NPVs were systematically slightly higher for UTI-related sepsis  
159 compared to intra-abdominal sepsis (Tables S2-S3, Figures S2-S3). NPV considering *intI1*  
160 alone gave comparable results: 92.0% [88.3-94.9].

161

162

## 163 Discussion

164 The IRIS multicentre study highlighted the utility of *intI1* and *intI2* for their NPVs.  
165 These results confirm previous data obtained from GNB-positive blood cultures.<sup>10</sup> The  
166 added-value here is that *intI* genes were detected directly from raw biological samples. This is  
167 a key point that can help with patient management right at admission. Even though several  
168 major ARGs of GNB are not cassette-borne genes (*bla*<sub>CTX-M-15</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>KPC...</sub>), the  
169 presence of *intI1* and/or *intI2* succeeded in predicting antibiotic susceptibilities. Information  
170 on the absence of *intI1* and *intI2* obtained in less than 2 hours could help clinicians in  
171 choosing 3GC for a non-severe septic patient at risk of MDR with a GNB-related infection.  
172 This would help preserving the use of carbapenems without compromising management. *intI1*

173 plus *intI2* constitute a biomarker that could be used as a complementary tool in therapeutic  
174 decisions. No other similar biomarker has been identified yet and is able to provide  
175 information on overall antibiotic susceptibility. This biomarker has some limits. First,  
176 integrons are scarcely described among Gram-positive bacteria. This limits the utility of the  
177 biomarker to GNB-related infections that need to be documented before detection of *intI*  
178 genes. Second, contrary to specific ARGs, *intI* genes cannot predict to what antibiotic the  
179 associated GNB will be resistant to.

180 *intI1* and/or *intI2* were detected in close to one third of patients (31.8%). Global and  
181 respective *intI1* plus *intI2* percentages, mainly driven by UTIs (29.6%), were very comparable  
182 to a single centre study performed on blood cultures (29.5%).<sup>10</sup> The percentage retrieved  
183 from intra-abdominal infections (43.1%) was also very comparable to a study focusing on  
184 stool samples (43.8%).<sup>17</sup> This higher percentage can be explained first by the presence of a  
185 more important number of GNB (and so a higher probability of finding *intI1* and/or *intI2*) and  
186 second by the fact that such patients had more healthcare-associated infections compared to  
187 patients with UTIs.

188 This study has one main limitation: the overall antibiotic resistance rate was somewhat  
189 moderate and we cannot apply our results to countries with higher levels of MDR.  
190 Nevertheless, the resistance profiles observed were comparable to the resistance profiles  
191 obtained from other French studies carried out in primary care,<sup>18</sup> in the community<sup>19</sup> or in  
192 patients below 65 years old.<sup>20</sup> This was in full agreement with most of our patients.

193  
194 The multicentre IRIS study demonstrated the utility of *intI1* plus *intI2* as a predictive  
195 marker to rule out AMR in patients with UTI or intra-abdominal infections. Regarding the  
196 extremely low prevalence of class 3 integrons in humans, we think *intI3* gene has to be left  
197 out of the assay for future studies. Further prospective impact studies with real-time detection  
198 of *intI1* and *intI2* are needed to assess their clinical relevance in daily antibiotic stewardship.

199

200

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205

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212

213

214 **Transparency Declaration**

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221

222

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- 272

273 **Table 1:** Baseline characteristics of the study patients, according to *int11-3* detection

Patients' characteristics*	<i>int11-3</i> genes		
	Negative (n = 277)	Positive (n = 129)	Total (n = 406)
<b>Sex</b>			
Female	147 (53.1)	68 (52.7)	215 (53.0)
Male	130 (46.9)	61 (47.3)	191 (47.0)
<b>Type of infection</b>			
Urinary	240 (86.6)	101 (78.3)	341 (84.0)
Intra-abdominal	37 (13.4)	28 (21.7)	65 (16.0)
<b>Type of sepsis</b>			
Sepsis	162 (58.5)	75 (58.1)	237 (58.4)
Severe sepsis	69 (24.9)	34 (26.4)	103 (25.4)
Septic shock	46 (16.6)	20 (15.5)	66 (16.3)
<b>Origin of infection</b>			
Community-acquired	232 (83.8)	98 (76.0)	330 (81.3)
Healthcare-associated	45 (16.2)	31 (24.0)	76 (18.7)
<b>Age</b>			
	64.3 (18.5)	65.2 (17.8)	64.7 (18.2)
	68.0 [55.0 ; 78.0]	69.0 [56.0 ; 79.0]	68.0 [55.0 ; 78.0]
<b>IGS2 score (n1 = 268, n2 = 127)</b>			
	30.8 (15.3)	31.1 (15.7)	31.0 (15.4)
	29.0 [21.0 ; 37.5]	27.0 [20.0 ; 38.0]	29.0 [21.0 ; 38.0]
<b>SOFA score (n1 = 271, n2 = 127)</b>			
	3.3 (3.7)	2.9 (3.3)	3.2 (3.6)
	2.0 [1.0 ; 5.0]	1.0 [0.0 ; 5.0]	2.0 [1.0 ; 5.0]

274 \*mean (standard deviation), median; (interquartile range); or — no. (%)

275

276 **Figure 1:** Simulation of negative (blue) and positive (red) predictive value of *intI1* and/or  
 277 *intI2* for multidrug resistance and resistance to clinically-relevant antibiotics.  
 278 A theoretical range of prevalence of resistance from 0% to 100% was used. Dashed line  
 279 represents the prevalence of resistance observed in the IRIS study.

