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Article

Occurrence of Extended Spectrum Beta-Lactamase Gram-Negative Bacteria from Non-Clinical Sources in Dubai, United Arab Emirates

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Supplementary Materials

Table S1. Biochemical characterization of 206 isolates

Sr. No	Code	Source	Microscopic Morphology	Gram Staining	Oxidase Test	Indole Test	MR Test	VP Test	Citrate Test
1	MUD15-01	Wastewater	Bacilli	-	-	+	+	-	-
2	MUD15-02	Wastewater	Coccobacilli	-	-	-	+	-	-
3	MUD15-03	Wastewater	Coccobacilli	-	-	-	+	-	-
4	MUD15-04	Wastewater	Bacilli	-	-	-	+	-	-
5	MUD15-05	Wastewater	Bacilli	-	+	-	-	+	+
6	MUD15-06	Wastewater	Bacilli	-	-	+	+	-	-
7	MUD15-07	Wastewater	Bacilli	-	-	+	+	-	-
8	MUD15-08	Wastewater	Coccobacilli	-	-	-	-	-	-
9	MUD15-09	Wastewater	Bacilli	-	+	-	+	-	-
10	MUD15-10	Wastewater	Bacilli	-	-	+	+	-	-
11	MUD15-11	Wastewater	Bacilli	-	-	+	+	-	-
12	MUD15-12	Wastewater	Bacilli	-	+	+	+	-	-
13	MUD15-13	Wastewater	Bacilli	-	+	-	-	+	+
14	MUD15-14	Wastewater	Bacilli	-	-	+	+	-	-
15	MUD15-15	Wastewater	Coccobacilli	-	-	-	-	-	-
16	MUD15-16	Wastewater	Coccobacilli	-	-	+	-	+	+
17	MUD15-17	Wastewater	Coccobacilli	-	-	-	-	+	+
18	MUD15-18	Wastewater	Coccobacilli	-	-	+	-	+	+
19	MUD15-19	Wastewater	Coccobacilli	-	+	+	-	+	+
20	MUD15-20	Wastewater	Coccobacilli	-	-	+	-	+	+
21	MUD15-21	Wastewater	Coccobacilli	-	-	+	-	+	+
22	MUD15-22	Wastewater	Coccobacilli	-	-	+	+	+	+
23	MUD15-23	Wastewater	Coccobacilli	-	-	+	-	+	+
24	MUD15-24	Wastewater	Coccobacilli	-	-	+	+	+	+
25	MUD15-25	Wastewater	Bacilli	-	-	+	-	+	+
26	MUD15-26	Wastewater	Coccobacilli	-	-	-	+	+	-
27	MUD15-27	Wastewater	Coccobacilli	-	-	-	+	-	+
28	MUD15-28	Beef	Bacilli	-	-	+	+	-	-
29	MUD15-29	Beef	Bacilli	-	-	-	+	-	-
30	MUD15-30	Beef	Coccobacilli	-	-	-	+	+	-

Sr. No	Code	Source	Microscopic Morphology	Gram Staining	Oxidase Test	Indole Test	MR Test	VP Test	Citrate Test
31	MUD15-31	Beef	Bacilli	-	-	-	+	-	+
32	MUD15-32	Beef	Bacilli	-	-	-	-	+	-
33	MUD15-33	Beef	Bacilli	-	-	-	+	-	-
34	MUD15-34	Beef	Bacilli	-	-	+	+	-	-
35	MUD15-35	Beef	Bacilli	-	-	+	+	-	-
36	MUD15-36	Beef	Bacilli	-	-	-	-	+	-
37	MUD15-37	Beef	Bacilli	-	-	+	+	-	-
38	MUD15-38	Beef	Bacilli	-	-	+	+	-	-
39	MUD15-39	Beef	Bacilli	-	-	+	+	-	-
40	MUD15-40	Lamb	Bacilli	-	-	+	+	+	-
41	MUD15-41	Lamb	Bacilli	-	-	+	+	-	-
42	MUD15-42	Lamb	Bacilli	-	-	+	+	-	-
43	MUD15-43	Lamb	Coccobacilli	-	-	+	+	-	+
44	MUD15-44	Lamb	Coccobacilli	-	-	-	+	-	-
45	MUD15-45	Wastewater	Bacilli	-	-	-	+	+	-
46	MUD15-46	Chicken	Coccobacilli	-	-	-	+	-	+
47	MUD15-47	Chicken	Bacilli	-	-	-	+	-	+
48	MUD15-48	Chicken	Coccobacilli	-	-	-	+	-	+
49	MUD15-49	Chicken	Bacilli	-	-	-	-	+	+
50	MUD15-50	Chicken	Bacilli	-	-	+	+	-	-
51	MUD15-51	Chicken	Bacilli	-	-	-	+	+	+
52	MUD15-52	Chicken	Bacilli	-	-	-	+	+	+
53	MUD15-53	Chicken	Coccobacilli	-	-	-	+	-	+
54	MUD15-54	Chicken	Bacilli	-	-	+	+	-	-
55	MUD15-55	Chicken	Bacilli	-	-	-	+	-	+
56	MUD15-56	Chicken	Coccobacilli	-	-	-	+	-	+
57	MUD15-57	Chicken	Bacilli	-	-	-	+	-	+
58	MUD15-58	Chicken	Bacilli	-	-	+	+	-	-
59	MUD15-59	Chicken	Bacilli	-	-	+	+	-	-
60	MUD15-60	Chicken	Coccobacilli	-	-	-	+	+	+
61	MUD15-61	Chicken	Coccobacilli	-	-	-	+	-	+
62	MUD15-62	Chicken	Coccobacilli	-	-	-	+	-	+
63	MUD15-63	Chicken	Coccobacilli	-	-	-	+	-	+

Sr. No	Code	Source	Microscopic Morphology	Gram Staining	Oxidase Test	Indole Test	MR Test	VP Test	Citrate Test
64	MUD15-64	Chicken	Bacilli	-	-	+	+	-	-
65	MUD15-65	Chicken	Bacilli	-	-	+	+	-	-
66	MUD15-66	Chicken	Bacilli	-	-	+	+	-	+
67	MUD15-67	Chicken	Bacilli	-	-	+	+	-	+
68	MUD15-68	Chicken	Bacilli	-	-	+	+	-	-
69	MUD15-69	Chicken	Bacilli	-	-	+	+	-	-
70	MUD15-70	Chicken	Bacilli	-	-	+	+	-	-
71	MUD15-71	Chicken	Bacilli	-	-	+	+	-	+
72	MUD15-72	Chicken	Coccobacilli	-	-	+	-	-	+
73	MUD15-73	Chicken	Coccobacilli	-	-	+	+	-	+
74	MUD15-74	Chicken	Bacilli	-	-	+	+	-	-
75	MUD15-75	Chicken	Bacilli	-	-	+	+	-	+
76	MUD15-76	Chicken	Bacilli	-	-	+	+	-	+
77	MUD15-77	Chicken	Bacilli	-	-	+	+	-	-
78	MUD15-78	Chicken	Bacilli	-	-	+	+	-	+
79	MUD15-79	Chicken	Bacilli	-	-	+	+	-	-
80	MUD15-80	Chicken	Bacilli	-	-	+	+	-	+
81	MUD15-81	Chicken	Bacilli	-	-	+	+	-	+
82	MUD15-82	Chicken	Bacilli	-	-	+	+	-	-
83	MUD15-83	Chicken	Bacilli	-	-	+	+	-	+
84	MUD15-84	Chicken	Bacilli	-	-	+	+	-	-
85	MUD15-85	Chicken	Bacilli	-	-	+	+	-	+
86	MUD15-86	Chicken	Bacilli	-	-	+	+	-	+
87	MUD15-87	Chicken	Bacilli	-	-	+	+	-	+
88	MUD15-88	Chicken	Bacilli	-	-	+	+	-	+
89	MUD15-89	Chicken	Bacilli	-	-	+	+	-	-
90	MUD15-90	Chicken	Bacilli	-	-	+	+	-	+
91	MUD15-91	Chicken	Bacilli	-	-	+	+	+	-
92	MUD15-92	Chicken	Bacilli	-	-	+	+	+	+
93	MUD15-93	Chicken	Bacilli	-	-	+	+	-	+
94	MUD15-94	Chicken	Bacilli	-	-	+	+	-	+
95	MUD15-95	Chicken	Bacilli	-	-	+	+	-	+
96	MUD15-96	Chicken	Bacilli	-	-	+	+	-	-

Sr. No	Code	Source	Microscopic Morphology	Gram Staining	Oxidase Test	Indole Test	MR Test	VP Test	Citrate Test
97	MUD15-97	Chicken	Bacilli	-	-	+	+	-	+
98	MUD15-98	Chicken	Bacilli	-	-	+	+	-	-
99	MUD15-99	Chicken	Bacilli	-	-	+	+	-	-
100	MUD15-100	Chicken	Bacilli	-	-	+	+	-	-
101	MUD15-101	Chicken	Bacilli	-	-	+	+	-	-
102	MUD15-102	Chicken	Bacilli	-	-	+	+	-	+
103	MUD15-103	Chicken	Bacilli	-	-	+	+	+	+
104	MUD15-104	Chicken	Bacilli	-	-	+	+	+	-
105	MUD15-105	Chicken	Bacilli	-	-	+	+	+	+
106	MUD15-106	Chicken	Bacilli	-	-	+	+	+	+
107	MUD15-107	Chicken	Bacilli	-	-	+	+	+	+
108	MUD15-108	Chicken	Bacilli	-	-	+	+	-	-
109	MUD15-109	Chicken	Bacilli	-	-	+	+	-	-
110	MUD15-110	Chicken	Bacilli	-	-	+	+	-	-
111	MUD15-111	Chicken	Bacilli	-	-	+	+	-	-
112	MUD15-112	Chicken	Bacilli	-	-	+	+	-	-
113	MUD15-113	Chicken	Bacilli	-	-	+	+	+	+
114	MUD15-114	Chicken	Bacilli	-	-	+	+	-	+
115	MUD15-115	Chicken	Bacilli	-	-	+	+	-	-
116	MUD15-116	Chicken	Bacilli	-	-	-	+	-	-
117	MUD15-117	Chicken	Bacilli	-	-	-	+	-	-
118	MUD15-118	Chicken	Bacilli	-	-	-	+	-	-
119	MUD15-119	Chicken	Bacilli	-	-	-	+	-	-
120	MUD15-120	Chicken	Bacilli	-	+	-	+	-	-
121	MUD15-121	Chicken	Bacilli	-	-	+	+	-	-
122	MUD15-122	Lamb	Bacilli	-	-	+	+	-	-
123	MUD15-123	Lamb	Bacilli	-	-	-	+	-	-
124	MUD15-124	Lamb	Bacilli	-	-	-	+	-	-
125	MUD15-125	Lamb	Bacilli	-	-	-	+	-	-
126	MUD15-126	Lamb	Bacilli	-	-	+	+	-	-
127	MUD15-127	Lamb	Bacilli	-	-	-	+	-	-
128	MUD15-128	Lamb	Bacilli	-	-	+	+	-	-
129	MUD15-129	Lamb	Bacilli	-	-	+	+	-	-

Sr. No	Code	Source	Microscopic Morphology	Gram Staining	Oxidase Test	Indole Test	MR Test	VP Test	Citrate Test
130	MUD15-130	Lamb	Bacilli	-	-	+	+	-	-
131	MUD15-131	Lamb	Coccobacilli	-	-	-	+	-	-
132	MUD15-132	Lamb	Bacilli	-	-	+	+	-	-
133	MUD15-133	Lamb	Bacilli	-	-	+	+	-	-
134	MUD15-134	Lamb	Coccobacilli	-	-	+	+	-	-
135	MUD15-135	Beef	Coccobacilli	-	-	-	+	-	-
136	MUD15-136	Beef	Bacilli	-	+	-	+	-	-
137	MUD15-137	Beef	Coccobacilli	-	-	-	+	-	-
138	MUD15-138	Beef	Coccobacilli	-	-	-	+	-	-
139	MUD15-139	Beef	Bacilli	-	-	-	+	-	-
140	MUD15-140	Beef	Coccobacilli	-	-	-	+	-	-
141	MUD15-141	Chicken	Bacilli	-	-	-	+	-	+
142	MUD15-142	Chicken	Bacilli	-	-	-	-	+	+
143	MUD15-143	Chicken	Bacilli	-	-	+	+	-	+
144	MUD15-144	Chicken	Bacilli	-	-	-	+	-	+
145	MUD15-145	Chicken	Bacilli	-	-	-	+	+	+
146	MUD15-146	Chicken	Bacilli	-	-	-	+	-	+
147	MUD15-147	Chicken	Coccobacilli	-	-	-	+	+	+
148	MUD15-148	Chicken	Bacilli	-	-	-	+	+	+
149	MUD15-149	Chicken	Coccobacilli	-	-	-	+	+	+
150	MUD15-150	Chicken	Bacilli	-	-	+	+	+	+
151	MUD15-151	Chicken	Bacilli	-	-	-	+	+	+
152	MUD15-152	Chicken	Bacilli	-	-	-	+	+	+
153	MUD15-153	Chicken	Bacilli	-	-	-	+	+	+
154	MUD15-154	Lamb	Bacilli	-	-	+	+	-	-
155	MUD15-155	Lamb	Bacilli	-	-	+	+	-	+
156	MUD15-156	Lamb	Bacilli	-	-	+	+	-	-
157	MUD15-157	Beef	Bacilli	-	-	+	+	-	-
158	MUD15-158	Beef	Bacilli	-	-	+	+	+	+
159	MUD15-159	Beef	Bacilli	-	-	+	+	+	+
160	MUD15-160	Beef	Bacilli	-	-	+	-	+	+
161	MUD15-161	Beef	Bacilli	-	-	+	+	+	+
162	MUD15-162	Beef	Bacilli	-	-	+	+	-	-

Sr. No	Code	Source	Microscopic Morphology	Gram Staining	Oxidase Test	Indole Test	MR Test	VP Test	Citrate Test
163	MUD15-163	Beef	Bacilli	-	-	+	+	+	+
164	MUD15-164	Beef	Bacilli	-	-	+	+	+	+
165	MUD15-165	Beef	Bacilli	-	-	+	+	-	-
166	MUD15-166	Beef	Bacilli	-	-	+	+	-	-
167	MUD15-167	Beef	Bacilli	-	-	+	-	-	+
168	ST - 01	Chicken	Coccobacilli	-	-	-	+	-	+
169	ST - 02	Chicken	Coccobacilli	-	-	-	+	-	+
170	ST - 03	Chicken	Coccobacilli	-	-	-	+	-	+
171	ST - 04	Chicken	Coccobacilli	-	-	-	+	-	-
172	ST - 05	Chicken	Coccobacilli	-	-	-	+	-	-
173	ST - 06	Chicken	Bacilli	-	-	+	+	-	-
174	ZU-01	Wastewater	Coccobacilli	-	+	-	+	-	-
175	ZU-02	Wastewater	Coccobacilli	-	-	-	+	-	-
176	ZU-03	Wastewater	Coccobacilli	-	-	-	-	-	+
177	ZU-04	Wastewater	Bacilli	-	+	-	+	-	+
178	ZU-05	Wastewater	Coccobacilli	-	+	-	+	-	+
179	ZU-06	Wastewater	Coccobacilli	-	+	-	+	-	+
180	ZU-07	Wastewater	Coccobacilli	-	+	-	+	-	+
181	ZU-08	Wastewater	Coccobacilli	-	+	-	+	-	+
182	ZU-09	Wastewater	Coccobacilli	-	+	-	+	-	+
183	ZU-10	Wastewater	Coccobacilli	-	-	-	+	-	-
184	ZU-11	Wastewater	Bacilli	-	-	+	+	-	+
185	ZU-12	Wastewater	Bacilli	-	-	+	-	-	+
186	ZU-13	Wastewater	Bacilli	-	-	+	-	-	+
187	ZU-14	Wastewater	Bacilli	-	+	-	-	-	+
188	ZU-15	Wastewater	Coccobacilli	-	-	-	+	-	+
189	ZU-16	Wastewater	Bacilli	-	-	+	+	-	-
190	ZU-17	Wastewater	Bacilli	-	+	-	+	-	+
191	ZU-18	Wastewater	Bacilli	-	-	+	+	-	-
192	ZU-19	Wastewater	Bacilli	-	-	+	+	-	+
193	ZU-20	Wastewater	Bacilli	-	-	-	+	-	+
194	ZU-21	Wastewater	Bacilli	-	-	+	+	-	-
195	ZU-22	Wastewater	Bacilli	-	-	+	+	-	-

Sr. No	Code	Source	Microscopic Morphology	Gram Staining	Oxidase Test	Indole Test	MR Test	VP Test	Citrate Test
196	ZU-23	Wastewater	Bacilli	-	+	-	+	-	+
197	ZU-24	Wastewater	Bacilli	-	-	+	+	-	-
198	ZU-25	Wastewater	Bacilli	-	+	-	-	-	+
199	ZU-26	Wastewater	Bacilli	-	-	-	+	-	+
200	ZU-27	Wastewater	Bacilli	-	-	-	+	-	+
201	ZU-28	Wastewater	Bacilli	-	-	-	+	+	+
202	ZU-29	Wastewater	Bacilli	-	+	+	+	-	+
203	ZU-30	Wastewater	Bacilli	-	-	+	-	-	+
204	ZU-31	Wastewater	Bacilli	-	-	-	+	-	+
205	ZU-32	Wastewater	Bacilli	-	-	+	-	-	+
206	ZU-33	Wastewater	Bacilli	-	-	+	+	-	+

Table S2. Antibiotics zones of inhibition diameter according to CLSI guidelines.

Antibiotic Code	Antibiotic	Potency (μg/disc)	Resistant (mm)	Intermediate (mm)*	Sensitive (mm)
CPD	Cefpodoxime	10	≤ 17	18-20	≥ 21
CAZ	Ceftazidime	30	≤ 17	18-20	≥ 21
CTX	Cefotaxime	30	≤ 22	23-25	≥ 26
CZX	Ceftizoxime	30	≤ 21	22-24	≥ 25

*Intermediate resistance is the partial resistance of a bacterium to a particular antibiotic that falls short of resistance.

Table S3. Susceptibility of *E.coli* ATCC 25922 to 3rd generation cephalosporins

Antibiotics	Code	CLSI Standard zone of Inhibition (mm)	Obtained Zone of Inhibition (mm)
Cefpodoxime	CPD [10]	23-28	24
Ceftazidime	CAZ [30]	25-32	28
Cefotaxime	CTX [30]	29-35	28
Ceftizoxime	CZX [30]	30-36	32

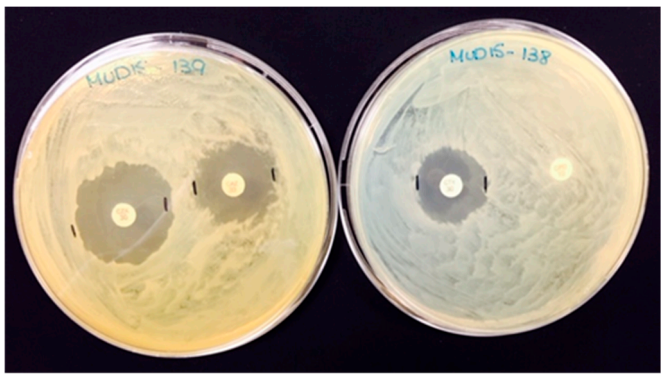

Figure S1. Antibiotic susceptibility assay with 3rd-generation cephalosporins: CPD, CAZ, CTX and CZX

Double disc diffusion test

The ESBL isolates were further confirmed by the Double Disc Diffusion Test according to the CLSI guidelines (Table S2) using the 4th generation Cephalosporin Cefpirome (CFP ^{30}) discs alone and in combination with Clavulanic acid (CPC $^{30/7.5}$). The isolates are confirmed as ESBLs if, after 24 hours incubation, the zone of inhibition in between the discs is enhanced (>5mm difference in diameter). *E.coli* ATCC 25922 was used as a control throughout the test.

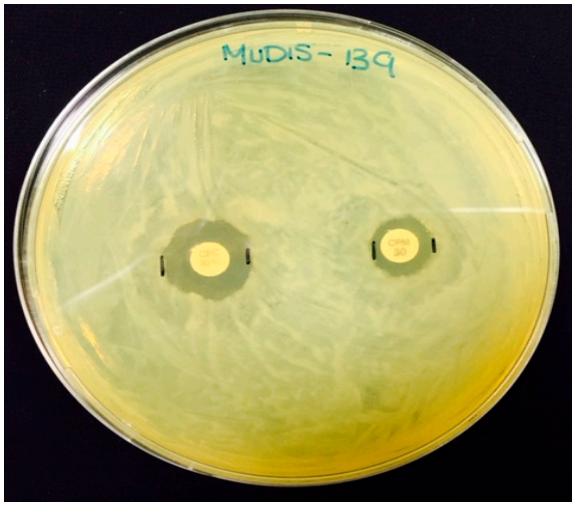

Figure S2. Confirmatory double disc diffusion test for ESBLs

Figure S3 shows that out of 86 potential ESBL isolates, 19 (22%) were confirmed as ESBL positive as they showed a zone of enhancement >5mm in diameter between the Cefpirome discs alone and in combination with Clavulanic acid.

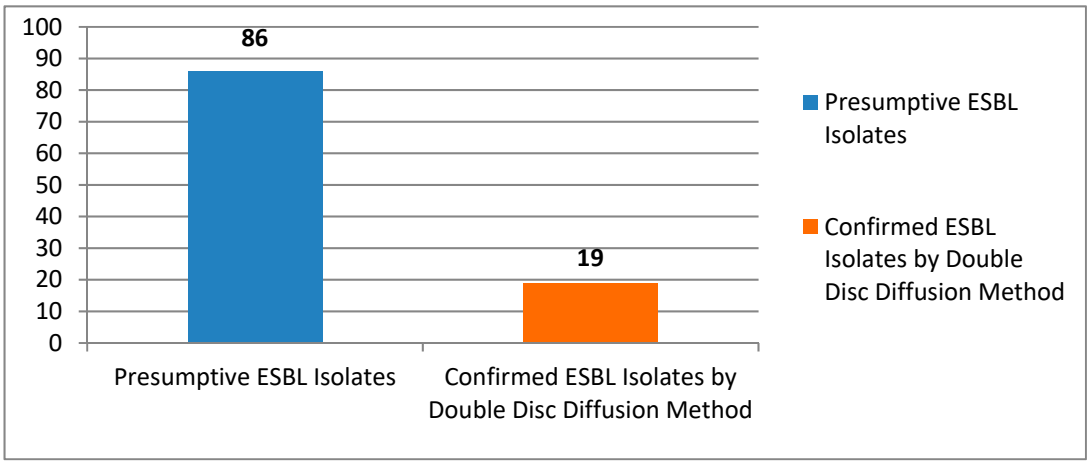

Figure S3. Number of potential and confirmed ESBL isolates by double disc diffusion method

Table S4. Susceptibility of *E.coli* ATCC 25922 to cefpirome

Antimicrobial agent	Code	CLSI Standard Zone of Inhibition (mm)	Obtained Zone of Inhibition (mm)
Cefpirome	CFP ^{30}	23-28	25

Combined Disc Test for Carbapenemase Resistance

The ESBL isolates were evaluated according to the CLSI guidelines (Table S2) by the Combined Disc Test which is based on the inhibition of MBL (Metallo-Beta-Lactamase) activity by EDTA. Two Imipenem discs, one containing Imipenem (IPM ^{10}) alone and the other containing Imipenem-EDTA (IE $^{10/750}$) were placed 25mm apart. An isolate producing a diameter of >4mm around the disc with IE and not around the disc with IPM alone was considered positive for MBL. *E.*coli ATCC 25922 was used as a control throughout the test.

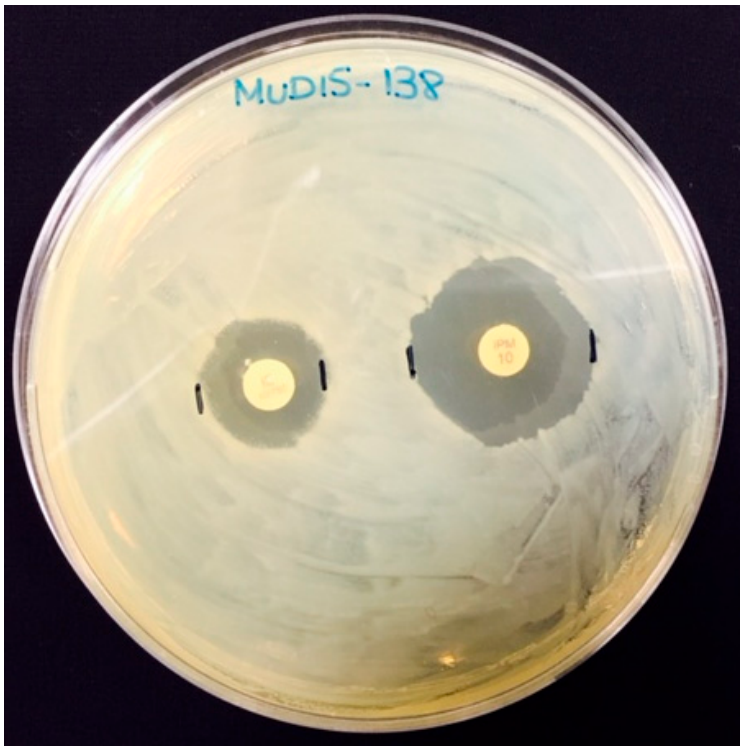

Figure S4. Zone of enhancement around imipenem plus EDTA

Figure S5 shows that out of 86 potential ESBL isolates, 8 (9.3%) were resistant to Imipenem and 1 (1%) MUD15-138 was confirmed as MBL positive as it showed a zone of enhancement >4mm in diameter between the Imipenem and Imipenem-EDTA discs.

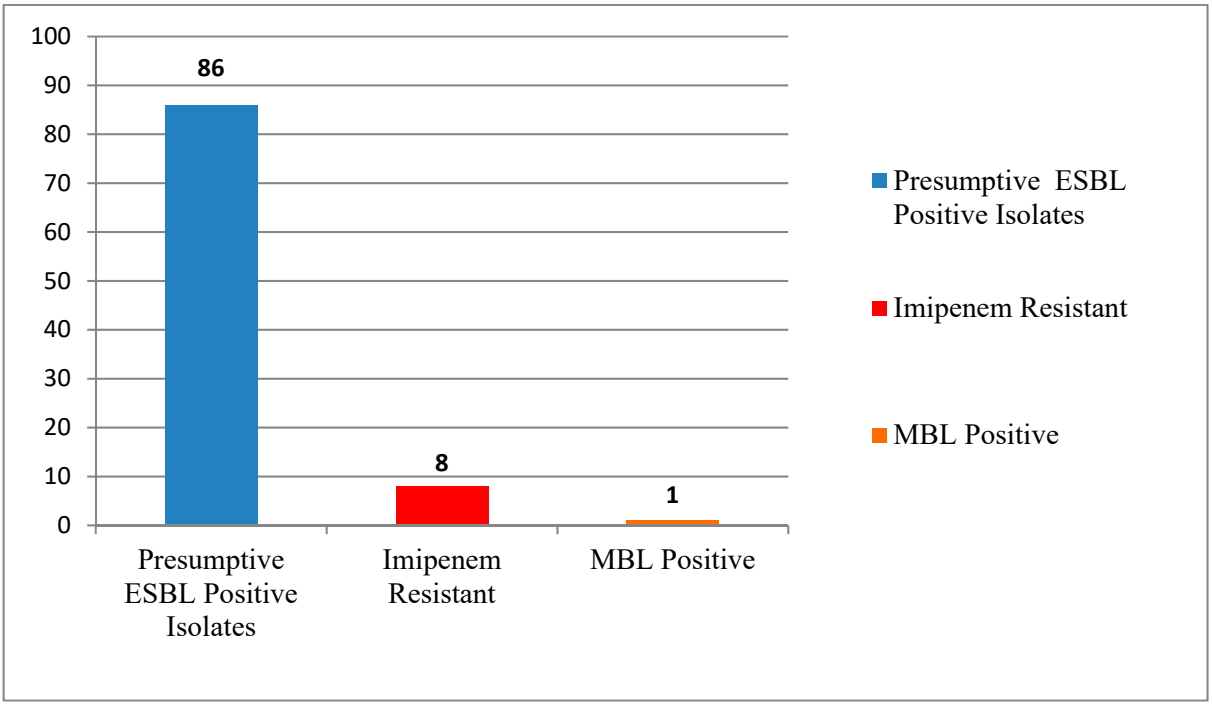

Figure S5. Number of imipenem resistant and MBL positive isolates

Table S5. Susceptibility of *E.*coli ATCC 25922 to imipenem

Antibiotic	Code	CLSI Standard Zone of Inhibition (mm)	Obtained Zone of Inhibition (mm)
Imipenem	IPM [10]	26-32	26