Profile of outer membrane proteins of carbapenem-resistant Gram-negative bacilli in Ghana

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Profile of outer membrane proteins of carbapenem-resistant Gram-negative bacilli in Ghana

Francis S. Codjoe[1,2], Fleischer C. N. Kotey[3] and Eric S. Donkor[3*]

Abstract

Objective Carbapenem resistance is a major global health threat, but insights on its molecular determinants are scanty in sub-Saharan Africa, the predominant global antimicrobial resistance hotspot. This study aimed to profile outer membrane proteins (OMPs) of 111 carbapenem-resistant Gram-negative bacteria recovered from a broad spectrum of clinical specimens from Ghana.

Results The OMPs of *Pseudomonas aeruginosa* produced decreased amounts of OprD or the porin was completely lost, except in 5.9% ($n=3$) of the isolates which had high-level porins. For *Acinetobacter baumannii*, 96.8% ($n=30$) expressed loss of OprF. One carbapenemase non-PCR-positive isolate with high-level porin expression was observed. In *A. baumannii*, the major and significant band on SDS-PAGE was ~35 kDa. There were substantial numbers of unrelated porin expression among the isolates. Particularly, OmpC/F or OmpK35/37 expression was deficient. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis for *Escherichia coli*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, and other Enterobacterales had OmpC and OmpF absent or markedly reduced compared to the control strains. Overall, porin loss was a major mechanism underlying carbapenem resistance among the isolates, suggesting that in carbapenem-resistant organisms that seem to lack known carbapenem resistance genes, porin loss may be the underlying carbapenem resistance mechanism.

Keywords Outer membrane proteins, OMPs, Porins, Carbapenem resistance, Gram-negative bacilli

Introduction

Carbapenem-resistant Gram-negative bacteria (CR-GNB) have emerged as major public health threats worldwide, leading to increased morbidity, mortality, and healthcare costs [1–3]. In sub-Saharan Africa, the predominant global hotspot for antimicrobial resistance (AMR), AMR surveillance studies have rarely evaluated carbapenem resistance, which has become prevalent in the region [4–6]. Most of the few that have done so merely reported on carbapenem resistance prevalence, with little information on the molecular basis of the resistance [7, 8]. Those that studied the molecular epidemiology of carbapenem resistance mainly focused on

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the occurrence of carbapenemase genes among the isolates [1–3]. The involvement of outer membrane proteins (OMPs) in the resistance traits, although important, have been largely overlooked in these studies.

OMPs, also known as porins, are major cell wall components of Gram-negative bacteria [9]. Among other things, they help maintain bacterial membrane integrity, facilitate adhesion to host mucosa, biofilm formation, and passage of molecules (including iron and antibiotics) across the bacterial cell membranes [10–13]. They are also crucial to perpetuation of AMR in Gram-negative bacteria, through gene expression- and structural rearrangement-induced reduced permeability [14–16]. Besides, owing to their abundance on the bacterial surface, outer membrane proteins could be exploited in the design of vaccines and novel and improved antimicrobials [17].

The goal of the current study was to characterise OMPs of CR-GNB recovered from a broad spectrum of 3,840 clinical specimens collected in a three-year multicentre AMR surveillance in Ghana [1]. We have previously reported on AMR profiles and distribution of carbapenemase genes [1], as well as genetic relatedness [2] among these isolates. Thus, this study adds an underexplored angle to the discussion on carbapenem resistance from an AMR-affected region where sparse insights on carbapenem resistance have emanated from.

Materials and methods

Details of the CR isolates and previous analyses

In total, 3,840 clinical specimens collected from four major hospitals (Volta Regional Hospital [in the Volta Region], AngloGold Mines Hospital [in the Ashanti Region], Effia-Nkwanta Hospital [in the Western Region], and the Korle Bu Teaching Hospital [in the Greater Accra Region]) were evaluated for AMR and screened for beta-lactamase and carbapenemase genes and beta-lactamase and carbapenemase production [1].

The specimens yielded 111 CR isolates, mainly *Pseudomonas aeruginosa* (51), *Acinetobacter baumannii* (31), *Escherichia coli* (12), *Pseudomonas putida* (7), *Klebsiella pneumoniae* (3), *Enterobacter cloacae* (3), and one each of *Sphingomonas paucimobilis*, *Shigella sonnei*, *Providencia stuartii*, and *Cronobacter sakazakii*; the OMP profiles of these were evaluated in this study.

Outer membrane protein analysis of the CR isolates

In total, 111 non-duplicated CR isolates from 55 male and 56 female patients aged between 3 days and 91 years were investigated. A recent study by Sugawara & Nikaido [18] claimed that the major proteins in the outer membrane of *Acinetobacter baumannii*, a non-fermenter isolate that belongs to the low-permeability trimeric porin group, but has been found to equate to *Escherichia coli* OmpF/OmpC, and also shares close homology to *Pseudomonas aeruginosa* OprF and *Escherichia coli* OmpA. Therefore, in-house MBL-positive (*VIM*-positive) and wild-type *Pseudomonas aeruginosa* control strains from the Sheffield Hallam University (SHU) collection were included and used for the study analysis of OMPs. Two methods, with some modifications, were employed separately for organisms in the Enterobacteriaceae family and *Acinetobacter* species [19], and for *Pseudomonas* species [20], due to structural differences. Briefly, bacterial cultures were grown aerobically at 37 °C in Mueller-Hinton broth for six hours, then harvested by centrifugation. The pellet was resuspended in phosphate-buffered saline with lysozyme, sonicated to disrupt the cells, and centrifuged multiple times to remove whole cells and cell debris. The supernatant was then centrifuged to pellet the cell membranes, which were resuspended in a detergent solution to extract the outer membrane vesicles. The OMPs were harvested by centrifugation, washed to remove the detergent, and stored at -20 °C for later use.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Resolving gels were made at a 12% concentration of acrylamide. Polymerisation of acrylamide was prepared by adding 16 µl N, N,N',N'-tetramethylethylenediamine (TEMED) (Bio-Rad); the gel solution was cast between two glass plates in the apparatus and then layered with isopropanol equilibrated with saturated aqueous sodium chloride. Once gel polymerisation was complete, the saturated isopropanol was removed and the area at the top of the gel was dried with filter paper. The stacking gels were made containing 6% acrylamide concentration. Acrylamide polymerisation was initiated by adding 10 µl TEMED; the gel solution was overlaid on the resolving gel, and 15-well comb was pushed into the gel to create sample wells. After complete polymerisation, the gel apparatus was assembled in the appropriate tank and covered in 1x SDS-PAGE running buffer made from 10x tris-glycine-SDS buffer in 500 ml of deionised water at pH 8.3; Tris base 15.0 g, Glycine 72.0 g, SDS 5.0 g. The crude OMP samples were mixed with SDS-PAGE loading buffer at a 6:1 ratio, boiled for 5–10 min, and loaded into the wells created in the gel. Electrophoresis was set at 130 volts per centimetre until the blue colour in the loading dye in the sample buffer had reached the end of the gel (approximately 45 to 60 min). OMPs were visualised by staining the gel for 1 h with Coomassie brilliant blue R solution, and then destained with destain solution overnight and 2 other changes of destain solution over 2 to 3 h until the gel was clear. OMP bands were compared with standard pre-stained protein ladder (Thermo-Scientific, Leicestershire, UK).

The analysis of the OMPs on SDS-PAGE of all the CR isolates were specifically compared with *Pseudomonas aeruginosa* control strains with or without OprD (~44 kDa) expression which affects susceptibility to carbapenems.

Ethical approval

The study was approved by the Ethical Committee of the School Biomedical & Allied Health Sciences, College of Health Sciences, University of Ghana (Identification Number: SAHS-ET/SAHS/PSM/ML/05/AA/26A/2012–2013). The data and the bacteria were analysed anonymously, and hence informed consent was not applicable.

Results

Results summary of previous investigations on the CR isolates

Among the 111 isolates, 58% ($n=64$) were meropenem-resistant, 66.7% ($n=74$) were imipenem-resistant, and 75% ($n=83$) were doripenem-resistant, with 58% ($n=64$) of the isolates having ≥ 32 mg/mL minimum inhibitory concentration (MIC) levels [1]. Also, 23.4% ($n=26$) of the isolates were demonstrated as carbapenemase-positive by polymerase chain reaction (PCR), and the highest carbapenemase gene prevalence was from *Acinetobacter* spp. (9 *NDM-1*-positives) and *P. aeruginosa* (2 postive for *NDM-1* and 7 positive for *VIM-1*), followed by *E. coli* (3 positive for *NDM-1*), *K. pneumoniae* (2 positive for *OXA-48*), one *VIM-1*-positive *P. putida* isolate, and one each *NDM-1*-positive *P. stuartii* and *S. sonnei*, respectively [1].

Porin expression in the CR isolates

There were different expressions of porins for all CR isolates investigated (Fig. 1). The OMPs of *Pseudomonas aeruginosa* produced decreased amounts of OprD or the porin was completely lost, except the 5.9% (3/51) with high levels of porin in them (Table 1). Most of the isolates which had loss of porins were among the presumptive carbapenemase non-PCR positives.

For *Acinetobacter baumannii*, 96.8% (30/31) expressed loss of OprF in them, which included the *NDM-1*-positive producers. One carbapenemase non-PCR-positive isolate with high-level expression of porin was observed. Of all the *Acinetobacter baumannii* isolates analysed, the major and significant band on SDS-PAGE was ~35 kDa.

There were substantial numbers of unrelated porin expression. In particular, OmpC/F or OmpK35/37 expression was deficient. SDS-PAGE analysis for *Escherichia coli*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, and others in the Enterobacteriaceae group had OmpC and OmpF absent or markedly reduced at Bands 35 kDa and 37 kDa, respectively, compared to control strains.

Discussion

This study profiled OMPs among a collection of carbapenem-resistant isolates from Ghana. OMPs of the *Pseudomonas aeruginosa* isolates produced decreased amounts of OprD or the porin was completely lost, except for 5.9% (3 out of 51) which had high levels of porin in them. The majority of the isolates which had loss of porins were among the presumptive carbapenemase non-PCRpositives. It is worth mentioning that the effect of OprD reduction or loss could be one of the mechanisms that explain the ease with which *Pseudomonas aeruginosa* is known to become resistant. High-level production of β-lactamase activity by nitrocefin assays were prevalent in all the CR isolates studied. The effect and presence of these non-plasmid determinant enzymes may contribute significantly to carbapenem resistance coupled with the loss of *OprD* gene [21].

Essentially, in most multidrug-resistant *Pseudomonas aeruginosa*, isolates chromosomally encode resistance mechanisms in association with hyperexpression of *AmpC*, overproduction of the *MexAB* efflux system, or both, without harbouring a carbapenem resistance gene that could inactivate the *OprD* gene. The effect of these resistance mechanisms and the presence of β-lactamase enzymes may have resulted in the high number of carbapenemase non-PCR positives. According to Yang et al. [22], porins play a significant role in increasing Gram-negative bacterial resistance to carbapenems. Importantly, presence of ESBL- or *AmpC*-type enzymes is an essential element for resistance among Enterobacteriaceae and non-fermenting *Pseudomonas* species. This recognition was also observed in CR isolates in this study. Thus, all the 7 *VIM-1*-producing *Pseudomonas aeruginosa* isolates were carriers of *TEM-1* and *SHV-1* enzymes; 6 carbapenemase non-PCR positives out of 7 *Pseudomonas putida* isolates including the only *VIM-1*-positive isolate had reduced OprD or loss of porin by the SDS-PAGE assessment in this study. Pirnay et al. [23] emphasised that the major determinant of resistance to imipenem, particularly, in *Pseudomonas aeruginosa* in the absence of MBL could be mutational change in the *oprD* gene. It is worth noting that most *Pseudomonas aeruginosa* carbapenemase non-PCR positives, including other CR isolates, showed decreased production of the *oprD* gene, which could be mutation-driven resistance. This phenomenon was observed on the electrophoretic patterns of the SDS-PAGE analysis with a reduction or loss of outer membrane porin D.

A study by Fusté et al. [24], also on multidrug-resistant *Pseudomonas aeruginosa*, revealed a similar reduction of the OprD, the main uptake of carbapenems, while in another study from Brazil [25], markedly reduced OprD expressions were observed in MBL-negative *Pseudomonas* isolates. This is in agreement with this present study,

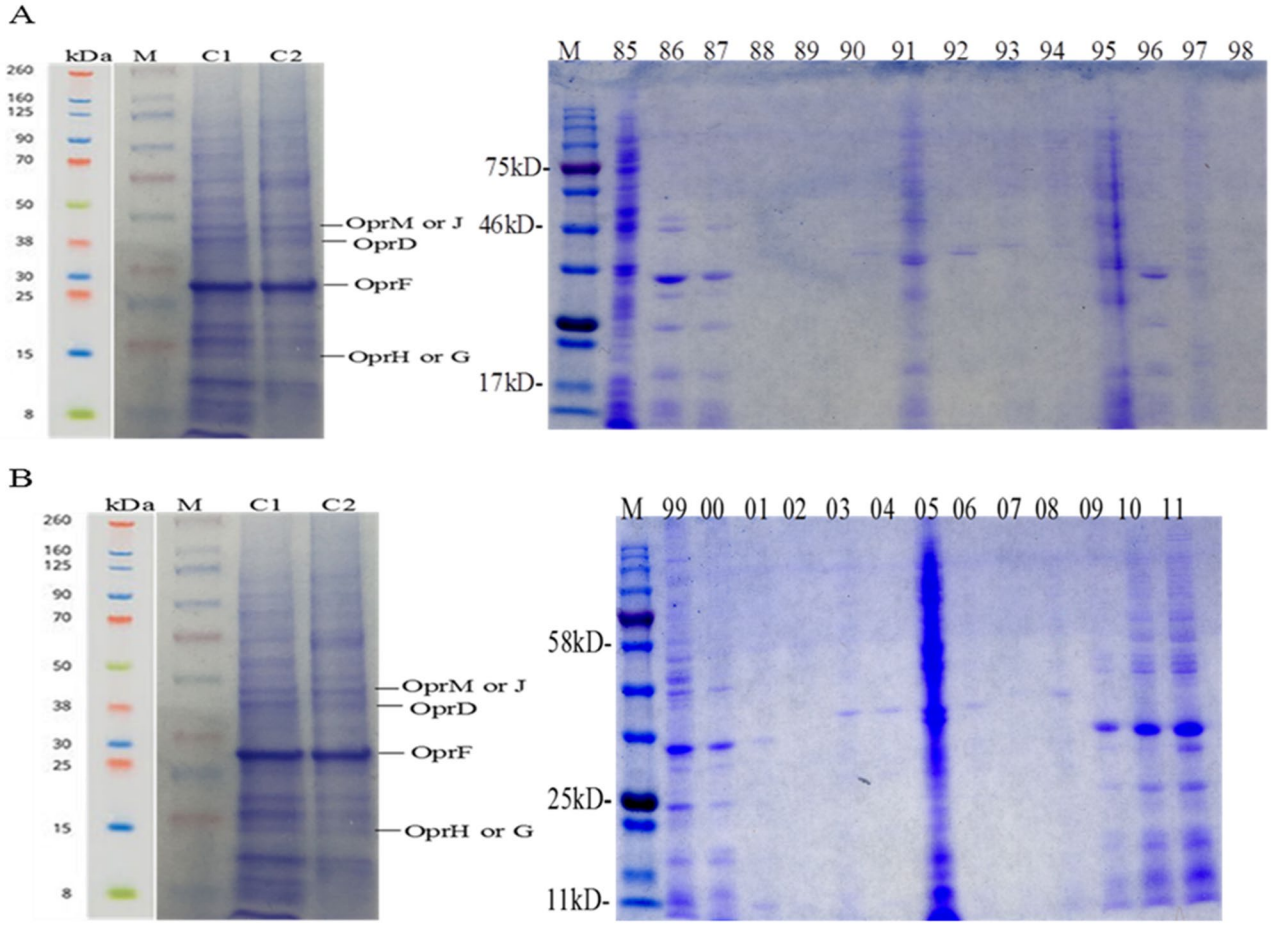

Fig. 1 Representative outer membrane proteins of control strains and carbapenem-resistant isolates on SDS-PAGE. Note M = Protein marker in kDa; C1 = Control SHU 2048 *VIM*-positive *Pseudomonas aeruginosa* strain; C2 = Control SHU 2054 MBL-negative *Pseudomonas aeruginosa* strain; 85–111 represent sample numbers; *Opr*M/J (48/49 kDa) = Major intrinsic multidrug efflux proteins; *Opr*D (44 kDa) = Specific for β-lactam imipenem resistance; *Opr*F (35 kDa) = Major porin for structural stabilisation of peptidoglycan; *Opr*H/G (22/23 kDa) = Polycationic antimicrobial uptake and putatively in iron uptake. Full-length gels are presented in Supplementary Fig. 1

which has demonstrated that OprD loss and VIM-1 production could be one of the possible mechanisms emerging in MBL negative *Pseudomonas aeruginosa* from Ghanaian hospitals.

For *Acinetobacter baumannii*, 96.8% expressed OprF loss, which included the *NDM-1*-positive producers. One carbapenemase non-PCR positive isolate with high-level porin expression was observed, although the mechanism of resistance remains unclear. It should be mentioned that the selectivity of the outer membrane permeability barrier is different for organisms such as *Acinetobacter* species or *Pseudomonas aeruginosa* compared with those in the Enterobacteriaceae group. The OmpAb of ~40 kDa, an OMP of *Acinetobacter baumannii* accounts for approximately 30–40% of the total OMPs [26], as compared to that of *Pseudomonas aeruginosa*. Li et al. [27] emphasised that these organisms do not produce the classical *Escherichia coli*-type trimeric porins that provide an increased influx of small drugs. The main nonspecific porin in these organisms is a homology of *Escherichia coli* OmpA, which functions by adding structural fitness usually connected to the outer membrane underlying peptidoglycan [27]. *Acinetobacter baumannii* has a close homology to *Escherichia coli* OmpA, an antibiotic selection and permeant porin [28] and *Pseudomonas aeruginosa* OprF, which is the major OMP [18]. Of all the *Acinetobacter baumannii* isolates, the major and significant band on SDS-PAGE was ~35 kDa, strongly suggesting that the outer membrane of *Acinetobacter baumannii* contains reduced amounts of low-permeability porins, a situation similar to the OprF of *Pseudomonas aeruginosa*.

The deficiency in *OmpC/F* or *OmpK35/37* expression could be due to mutation and the presence of a large collection of non-fermenters highly resistant to cephalosporins and carbapenems studied, mainly, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates. These organisms have mutational complexities, structural

Table 1 Carbapenem MIC levels and major porins in carbapenem-resistant isolates

Isolate Code	Name of isolate	A	B	C	Major porins	
					OprD[a]	OprF[b]
1	*P. aeruginosa*	+	–	–	H	M
4	*A. baumannii*	+	–	–	–	–
6*	*P. aeruginosa*	+	–	–	R	–
7*	*P. aeruginosa*	+	–	–	–	–
8	*A. baumannii*	+	–	–	–	–
9	*C. sakazakii*	+	–	–	–	–
10	*P. aeruginosa*	+	–	–	–	–
11	*S. paucimobilis*	+	–	–	–	–
12*	*P. putida*	+	–	–	–	–
16	*P. aeruginosa*	+	–	–	R	M
20	*A. baumannii*	+	–	–	–	–
23	*E. coli*	+	–	–	–	–
24	*P. aeruginosa*	+	–	–	–	R
25	*P. aeruginosa*	+	–	–	–	R
26	*P. aeruginosa*	+	–	–	–	–
29	*A. baumannii*	+	–	–	–	–
32	*P. aeruginosa*	+	–	–	–	R
33	*En. Cloacae*	+	–	–	–	–
36	*A. baumannii*	+	–	–	–	–
37	*P. aeruginosa*	+	–	–	R	–
38	*A. baumannii*	+	–	–	–	–
39*	*P. aeruginosa*	+	–	–	–	–
40	*P. aeruginosa*	+	–	–	R	R
41	*P. aeruginosa*	+	–	–	R	–
42*	*A. baumannii*	+	–	–	R	–
43*	*A. baumannii*	+	–	–	–	–
44	*P. putida*	+	–	–	–	–
45	*A. baumannii*	+	–	–	–	–
46*	*A. baumannii*	+	–	–	–	–
47*	*P. aeruginosa*	+	–	–	–	–
48	*P. aeruginosa*	+	–	–	–	–
49	*A. baumannii*	+	–	–	–	–
51	*A. baumannii*	+	–	–	–	–
53	*E. coli*	+	–	–	–	–
56	*A. baumannii*	+	–	–	M	M
60*	*P. aeruginosa*	+	–	–	R	–
61	*P. aeruginosa*	+	–	–	–	–
62*	*P. aeruginosa*	+	–	–	R	–
63	*P. aeruginosa*	+	–	–	–	–
64*	*A. baumannii*	+	–	–	–	–
65	*P. aeruginosa*	+	–	–	–	–
72*	*A. baumannii*	+	–	–	–	–
74*	*P. aeruginosa*	+	–	–	–	M
75	*A. baumannii*	+	–	–	–	–
77	*A. baumannii*	+	–	–	–	–
78	*P. putida*	+	–	–	–	–
79	*P. aeruginosa*	+	–	–	–	M
82	*A. baumannii*	+	–	–	R	–
86	*P. aeruginosa*	+	–	–	–	H
87	*P. aeruginosa*	+	–	–	–	M
88	*P. putida*	+	–	–	–	–
90	*P. putida*	+	–	–	–	–

Table 1 (continued)

91	*En. Cloacae*	+	–	–	–	R
92	*P. putida*	+	–	–	–	–
93	*E. coli*	+	–	–	–	R
95	*En. Cloacae*	+	–	–	R	R
97	*E. coli*	+	–	–	–	–
100*	*P. aeruginosa*	+	–	–	–	R
102*	*A. baumannii*	+	–	–	–	–
106	*A. baumannii*	+	–	–	R	–
108*	*A. baumannii*	+	–	–	R	–
109	*P. aeruginosa*	+	–	–	R	–
110	*P. aeruginosa*	+	–	–	R	H
2*	*E. coli*	–	+	–	R	–
13	*P. aeruginosa*	–	+	–	–	–
14	*P. aeruginosa*	–	+	–	–	+
15	*P. aeruginosa*	–	+	–	R	R
17	*P. aeruginosa*	–	+	–	–	–
18	*E. coli*	–	+	–	–	–
19*	*K. pneumoniae*	–	+	–	–	–
22	*A. baumannii*	–	+	–	–	–
27*	*A. baumannii*	–	+	–	–	–
28	*P. aeruginosa*	–	+	–	R	H
30	*A. baumannii*	–	+	–	–	–
31	*P. aeruginosa*	–	+	–	R	–
34	*A. baumannii*	–	+	–	–	–
50*	*A. baumannii*	–	+	–	–	–
52	*P. aeruginosa*	–	+	–	–	–
54	*P. aeruginosa*	–	+	–	–	–
55	*K. pneumoniae*	–	+	–	–	–
57	*P. aeruginosa*	–	+	–	–	–
58	*P. aeruginosa*	–	+	–	–	–
66	*P. aeruginosa*	–	+	–	–	–
69	*E. coli*	–	+	–	–	–
71	*P. aeruginosa*	–	+	–	R	–
73	*A. baumannii*	–	+	–	–	–
80	*P. aeruginosa*	–	+	–	–	–
84*	*P. aeruginosa*	–	+	–	–	–
94	*A. baumannii*	–	+	–	–	–
96	*P. aeruginosa*	–	+	–	–	–
98*	*K. pneumoniae*	–	+	–	–	–
99	*P. aeruginosa*	–	+	–	M	H
101	*A. baumannii*	–	+	–	–	–
104	*En. Cloacae*	–	+	–	R	–
107	*A. baumannii*	–	+	–	–	–
111	*P. aeruginosa*	–	+	–	R	H
3	*P. aeruginosa*	–	–	+	R	–
5*	*E. coli*	–	–	+	R	–
21	*P. aeruginosa*	–	–	+	–	R
35	*E. coli*	–	–	+	–	–
59	*P. aeruginosa*	–	–	+	M	M
67	*E. coli*	–	–	+	–	–
68	*E. coli*	–	–	+	H	–
70	*P. aeruginosa*	–	–	+	R	–
76*	*P. stuartii*	–	–	+	–	–
81	*P. aeruginosa*	–	–	+	–	H

Table 1 (continued)

83	*P. putida*	–	–	+	M	–
85	*E. coli*	–	–	+	H	–
89*	*S. sonnei*	–	–	+	–	–
103	*A. baumannii*	–	–	+	R	–
105*	*E. coli*	–	–	+	H	–

*Positive carbapenemase producers

[a] Analysis mainly for *Pseudomonas* species only

[b] Analysis for all CR isolates

Note: A = 3 carbapenems (imipenem, meropenem, ertapenem) (E-test levels were ≥ 32 µg/ml [*n* = 63]); B = 1 or 2 carbapenems (imipenem, meropenem, ertapenem) (E-test levels were between ≥ 16 and ≤ 4 µg/ml [*n* = 33]); C = 1 or 2 carbapenems (imipenem, meropenem, ertapenem) (E-test levels were < 4 µg/ml [*n* = 15]), + = Susceptible; – = Negative; H = High level of protein; M = Moderate level of protein; R = Low/reduced level of protein

differences, diminishing permeability or altered porin expressions compared to *Escherichia coli* and *Klebsiella pneumoniae* which were relatively few in numbers in this study.

Other membrane proteins were analysed and also compared with the *Pseudomonas aeruginosa* control strains since the porins are closely related to the absence or presence of OmpA, OmpAb, OmpC, and OmpF porins which have similar resistance to β-lactams and other antimicrobials. The SDS-PAGE analysis for *Escherichia coli*, *Klebsiella aerogenes*, *Klebsiella pneumoniae*, and others in the Enterobacteriaceae group had OmpC and OmpF absent or markedly reduced at Bands 35 kDa and 37 kDa, respectively, compared to control strains. The presence of OmpA in most of the Enterobacteriaceae group, for instance, in *Klebsiella aerogenes*, is the major source of stabilisation within the bacterial membrane, which leads to a reduction in the expression of OmpC and OmpF porins, and eventually a reduced susceptibility to β-lactams and other antimicrobials [29, 30]. Previous studies explained that the level of expression of OmpC and OmpF porins may not necessarily control the permeability of the outer membrane to nitrogen and glucose uptake under nutrient restriction, but may also be due to differential control by the concentration of certain antimicrobials within the environment [31, 32]. These studies are in agreement with this present study analysis which accounted for the high number of carbapenemase non-PCR positives.

Limitation

The study is limited by the few number of isolates evaluated and the age of the data.

Conclusion

In all, porin loss was demonstrated as one of the possible major mechanisms underlying the carbapenem resistance trait of the investigated isolates. Thus, in carbapenem-resistant organisms that do not seem to harbour known carbapenem resistance genes, porin loss may be the underlying carbapenem resistance mechanism.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-024-07070-6.

Supplementary Material 1

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Author contributions

Conceptualisation, F.S.C. and E.S.D.; methodology, F.S.C., F.C.N.K., and E.S.D.; software, F.S.C.; validation, E.S.D. and F.S.C.; formal analysis, F.S.C., F.C.N.K., and E.S.D.; investigation, F.S.C., F.C.N.K., and E.S.D.; resources, E.S.D. and F.S.C.; data curation, F.S.C., E.S.D., and F.C.N.K.; writing—original draft preparation, F.S.C., E.S.D., and F.C.N.K.; writing—review and editing, F.S.C., E.S.D., and F.C.N.K.; visualisation, F.S.C., E.S.D., and F.C.N.K.; supervision, E.S.D.; project administration, F.S.C. and E.S.D.; funding acquisition, F.S.C. and E.S.D. All authors have read and agreed to the published version of the manuscript.

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Data availability

The datasets used and/or analysed during the current study are not publicly available due to ethical restrictions, but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of the School Biomedical & Allied Health Sciences, College of Health Sciences, University of Ghana (Identification Number: SAHS-ET/SAHS/PSM/ML/05/AA/26A/2012–2013). Consent to participate was not applicable in this study, as the studied bacteria and their associated dataset had been anonymised.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Codjoe FS, Donkor ES, Smith TJ, Miller K. (2019). Phenotypic and genotypic characterization of carbapenem-resistant gram-negative bacilli pathogens from hospitals in Ghana. *Microbial Drug Resistance*, 25 (10):1449–1457. Online: http://login.research4life.org/tacsgrhttps://doi.org/10.1089/mdr.2018.0278. doi: 10.1089/mdr.2018.0278.
2. Codjoe FS, Brown CA, Smith TJ, Miller K, Donkor ES. Genetic relatedness in carbapenem-resistant isolates from clinical specimens in Ghana using ERIC-PCR technique. PLoS ONE. 2019;14(9):e0222168. https://doi.org/10.1371/journal.pone.0222168.
3. Dwomoh FP, Kotey FCN, Dayie NTKD, et al. Phenotypic and genotypic detection of carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Accra, Ghana. PLoS ONE. 2022;17(12):e0279715. https://doi.org/10.1371/journal.pone.0279715.
4. Kaonga N, Hang'ombe BM, Lupindu AM, Hoza AS. (2021). Detection of CTX-M-type extended-spectrum beta-lactamase-producing *Salmonella* Typhimurium in commercial poultry farms in Copperbelt Province, Zambia. 1 (2):27–34. https://doi.org/10.51585/gjvr.2021.2.001
5. Njeru J. Emerging carbapenem resistance in ESKAPE organisms in sub-saharan Africa and the way forward. Ger J Microbiol. 2021;1(1):3–6. https://doi.org/10.51585/gjm.2021.002.
6. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022. https://doi.org/10.1016/S0140-6736(21)02724-0.
7. Jean S, Harnod D, Hsueh P. Global threat of carbapenem-resistant Gram-negative bacteria. Front Cell Infect Microbiol. 2022;12:823684. https://doi.org/10.3389/fcimb.2022.823684.
8. Rima M, Oueslati S, Dabos L, Daaboul D, Mallat H, Raad B, E., et al. Prevalence and molecular mechanisms of carbapenem resistance among Gram-negative bacilli in three hospitals of Northern Lebanon. Antibiotics. 2022;11(10):1295.
9. Dehghani B, Mottamedifar M, Khoshkharam-Roodmajani H, Hassanzadeh A, Zomorrodian K, Rahimi A. SDS-PAGE analysis of the outer membrane proteins of uropathogenic *Escherichia coli* isolated from patients in different wards of Nemazee Hospital, Shiraz, Iran. Iran. J Med Sci. 2016;41:399–405.
10. Knopp M, Andersson DI. Amelioration of the fitness costs of antibiotic resistance due to reduced outer membrane permeability by upregulation of alternative Porins. Mol Biol Evol. 2015;32(12):3252–63.
11. Wurpel DJ, Moriel DG, Totsika M, Easton DM, Schembri MA. Comparative analysis of the uropathogenic *Escherichia coli* surface proteome by tandem mass-spectrometry of artificially induced outer membrane vesicles. J Proteom. 2015;115:93–106.
12. Vergalli J, Bodrenko IV, Masi M, Moynie L´ S, Acosta-Gutı´errez JH, Naismith A, Davin-Regli M, Ceccarelli B, van den Berg M, Winterhalter JM, Page`s. Porins and small-molecule translocation across the outer membrane of gram-negative bacteria. Nat Rev Microbiol. 2020;18(3):164–76.
13. Uppalapati SR, Sett A, Pathania R. The outer membrane proteins OmpA, CarO, and OprD of *Acinetobacter baumannii* confer a twopronged defense in facilitating its success as a potent human pathogen. Front Microbiol. 2020;11:589234. https://doi.org/10.3389/fmicb.2020.589234.
14. Ghai I, Ghai S. Understanding antibiotic resistance via outer membrane permeability. Infect Drug Resist. 2018;11:523–30. https://doi.org/10.2147/IDR.S156995.
15. Yang SK, Yusoff K, Thomas W, Akseer R, Alhosani MS, Abushelaibi A, Lim SHE, Lai KS. Lavender essential oil induces oxidative stress which modifies the bacterial membrane permeability of carbapenemase producing *Klebsiella pneumoniae*. Sci Rep. 2020;10:1–14. https://doi.org/10.1038/s41598-019-55601-0.
16. Maiti B, Chakraborty A, Karunasagar I. Biotechnological advances in the development of outer membrane protein-based vaccines for use in aquaculture. In: Gupta S, editor. Biotechnological advances in aquaculture health management. Singapore: Springer; 2021. pp. 43–59.
17. Alteri C, Mobley HLT. Quantitative Profile of the uropathogenic *Escherichia coli* outer membrane proteome during growth in human urine. Infect Immun. 2007;75:2679–88.
18. Sugawara E, Nikaido H. OmpA is the principal nonspecific slow porin of *Acinetobacter baumannii*. J Bacteriol. 2012;194(15):4089–96.
19. Mizuno T, Kageyama M. Separation and characterization of the outer membrane of *Pseudomonas aeruginosa*. J BioChem. 1978;84(1):179–91.
20. Sambrook J, Russell DW. (2001). Molecular cloning: A laboratory manual. 3rd. New York, Cold spring harbour laboratory press.
21. Mathlouthi N, Areig Z, Al Bayssari C, Bakour S, Salabi E, Gwierif AA, Zorgani SB, Slama AA, Chouchani KB, C. and, Rolain JM. Emergence of carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates collected from some Libyan hospitals. Microb Drug Resist. 2015;21(3):335–41.
22. Yang FC, Yan JJ, Hung KH, Wu JJ. Characterization of ertapenem-resistant *Enterobacter cloacae* in a Taiwanese university hospital. J Clin Microbiol. 2012;50(2):223–6.
23. Pirnay JP, De VD, Mossialos D, Vanderkelen A, Cornelis P, Zizi M. Analysis of the *Pseudomonas aeruginosa oprD* gene from clinical and environmental isolates. Environ Microbiol. 2002;4(12):872–82.
24. Fusté E, López-Jiménez L, Segura C, Gainza E, Vinuesa T, Viñas M. Carbapenem-resistance mechanisms of multidrug-resistant *Pseudomonas aeruginosa*. J Med Microbiol. 2013;62(Pt 9):1317–25.
25. Cavalcanti FL, Mirones CR, Paucar ER, Montes LÁ, Leal-Balbino TC, Morais MM, Martínez-Martínez L, Ocampo-Sosa AA. Mutational and acquired carbapenem resistance mechanisms in multidrug resistant *Pseudomonas aeruginosa* clinical isolates from Recife, Brazil. Memórias do Instituto Oswaldo Cruz. 2015;110(8):1003–9.
26. Jyothisri K, Deepak V, Rajeswari MR. Purification and characterization of a major 40 kDa outer membrane protein of *Acinetobacter baumannii*. FEBS Lett. 1999;443(1):57–60.
27. Li XZ, Plésiat P, Nikaido H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clin Microbiol Rev. 2015;28(2):337–418.
28. Iyer R, Moussa SH, Durand-Reville TF, Tommasi R, Miller A. Acinetobacter baumannii OmpA is a selective antibiotic permeant porin. ACS Infect Dis. 2018;4:373–81. https://doi.org/10.1021/acsinfecdis.7b00168.
29. Dupont M, De E, Chollet R, Chevalier J, Pagès JM. Enterobacter aerogenes OmpX, a cation-selective channel mar- and osmo-regulated. FEBS Lett. 2004;569(1–3):27–30.
30. Amaral L, Martins A, Spengler G, Molnar J. Efflux pumps of Gram-negative bacteria: what they do, how they do it, with what and how to deal with them. Front Pharmacol. 2014;4(168):1–11.
31. Randall LP, Woodward MJ. The multiple antibiotic resistance (mar) locus and its significance. Res Vet Sci. 2002;72(2):87–93.
32. Castillo-Keller M, Vuong P, Misra R. Novel mechanism of *Escherichia coli* porin regulation. J Bacteriol. 2006;188(2):576–86.

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