Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against Gram-negative multidrug-resistant bacteria

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Abstract

Background: Many edible plants are used in Cameroon since ancient time to control microbial infections. This study was designed at evaluating the antibacterial activities of the methanol extracts of ten Cameroonian vegetables against a panel of twenty nine Gram negative bacteria including multi-drug resistant (MDR) strains.

Methods: The broth microdilution method was used to determine the Minimal Inhibitory Concentrations (MIC) and the Minimal Bactericidal Concentrations (MBC) of the studied extracts. When chloramphenicol was used as a reference antibiotic, the MICs were also determined in the presence of Phenylalanine-Arginine β-Naphtylamide (PAβN), an efflux pumps inhibitor (EPI). The phytochemical screening of the extracts was performed using standard methods.

Results: All tested extracts exhibited antibacterial activities, with the MIC values varying from 128 to 1024 mg/L. The studied extracts showed large spectra of action, those from L. sativa, S. edule, C. pepo and S. nigrum being active on all the 29 bacterial strains tested meanwhile those from Amaranthus hybridus, Vernonia hymenolepis, Lactuca carpensis and Manihot esculenta were active on 96.55% of the strains used. The plant extracts were assessed for the presence of large classes of secondary metabolites: alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, steroids, tannins and triterpenes. Each studied plant extract was found to contain compounds belonging to at least two of the above mentioned classes.

Conclusion: These results confirm the traditional claims and provide promising baseline information for the potential use of the tested vegetables in the fight against bacterial infections involving MDR phenotypes.

Keywords: Antibacterial, Gram-negative bacteria, Multi-drug resistant, Extract, Vegetable

Background

Infectious diseases are still a major health concern, accounting for 41% of the global disease burden measured in terms of Disability-Adjusted Life Years (DALYS), close to all noninfectious diseases (43%) and far more than injuries (16%) [1]. One of the main causes of this problem is the widespread emergence of acquired bacterial resistance to antibiotics in such a way that the world is facing today, a serious threat to global public health [2] in the form of not only epidemics, but also pandemics of antibiotic resistance [3]. Several mechanisms have been accounted for, but active efflux plays an important role in this phenomenon [4]. The accumulation of different antibiotic resistance mechanisms within the same strains has led to the appearance of the so called superbugs, or multi-drug resistant bacteria [2]. Due to this problem of resistance to antibiotics, attention is now being shifted towards biologically active components isolated from plant species commonly used as herbal medicine, as they may offer a new source of antibacterial, antifungal and antiviral activities [5]. The potential antimicrobial properties of plants are related to their ability to synthesize several secondary metabolites of relatively complex structures possessing antimicrobial activities [6,7]. Among medicinal plants, vegetables associated to non or less-toxic effects have been shown to possess many medicinal properties [8,9] including antibacterial effects [3]. The present work was therefore designed to investigate the antibacterial effects of ten...
Cameroonian vegetables namely *Amaranthus hybridus* Linn (Amarantaceae), *Vernonia hymenolepis* (H.F.) Hook, *Lactuca sativa* Linn. and *Lactuca capensis* Thumb. (Asteraceae), *Manihot esculenta* Crantz (Euphorbiaceae), *Phaseolus vulgaris* Linn (Fabaceae), *Cucurbita pepo* Linn and *Sechium edule* (Jacq) Sw. (Cucurbitaceae), *Solanium nigrum* Linn. and *Capsicum frutescens* L. (Solanaceae) against MDR bacteria expressing active efflux pumps

**Methods**

**Plant material and extraction**

The collected plant materials used in this study were harvested from Dschang, West Region of Cameroon in June 2010 and included the leaves of *Amaranthus hybridus*, *Vernonia hymenolepis*, *Lactuca sativa*, *Lactuca capensis*, *Sechium edule*, *Manihot esculenta*, *Cucurbita pepo*, *Solanium nigrum*, the cloues of the Green bean (*Phaseolus vulgaris*), and the fruits of *Capsicum frutescens*. These plants were identified by Mr Victor Nana of the National Herbarium (Yaoundé-Cameroon) where all the voucher specimens were deposited with the corresponding reference number (Table 1).

Air dried and powdered sample (1 kg) of each plant was extracted with methanol (MeOH) for 48 h at room temperature (25°C), using Whatman Grade No.1 filter paper and concentrated under reduced pressure, then dried to give the crude extracts. All extracts were stored at 4°C until further use.

**Preliminary phytochemical investigations**

The major secondary metabolites classes such as alkaloids, anthocyanins, anthaquinones, flavonoids, phenols, saponins, tannins, sterols and triterpenes were screened according to the common phytochemical methods previously described by Harbone, 1973 [70].

**Bacterial strains and culture media**

The studied bacteria included both reference (from the American Type Culture Collection) and clinical strains of *Providencia stuartii*, *Pseudomonas aeruginosa*, *K. pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes* and *Enterobacter cloacae* (See Additional file 1: Table S1 for their features). These clinical strains were obtained from the laboratory “Transporteurs Membranaires, Chimiorésistance et Drug Design, UMR-MD1, IFR 88, UFRs de Médecine et de Pharmacie, Marseille, France”.

All strains were maintained in Nutrient Broth at 4°C and activated on Mueller Hinton Agar plates 24 h prior to any antimicrobial test. Mueller Hinton Broth (MHB) was used for all antibacterial assays.

**Bacterial susceptibility testing**

The MICs were determined using the rapid INT colorimetric assay [71,72]. Briefly, test samples were first emulsified in DMSO/MHB (50:50 V/V). The solution obtained was then added to MHB, and serially diluted two fold (in a 96- wells microplate). One hundred microlitres (100 μl) of inoculum (1.5 × 10⁶ CFU/ml) prepared in MHB was then added. The plate was covered with a sterile plate sealer, then agitated to mix the contents of the wells using a shaker and incubated at 37°C for 18 h. The final concentration of DMSO was 2.5% and did not affected the microbial growth. Wells containing MHB, 100 μl of inoculum and DMSO at a final concentration of 2.5% served as negative control. The MICs of samples were detected after 18 h incubation at 37°C, following addition of 40 μl of a 0.2 mg/ml INT solution and incubation at 37°C for 30 minutes. Viable bacteria reduce this yellow dye to pink. MIC was defined as the lowest sample concentration that exhibited complete inhibition of microbial growth and then prevented this change [73]. The MBC was determined by adding 50 μl of the suspensions from the wells, which did not show any growth after incubation during MIC assays, to 150 μl of fresh broth. These suspensions were re-incubated at 37°C for 48 hours. The MBC was determined as the lowest concentration of extract which completely inhibited the growth of bacteria [74]. Chloramphenicol, used as reference antibiotic, was tested also in the presence of the PAβN, at 30 mg/L final concentration to confirm the resistance of bacterial strains.

**Results**

**Chemical composition of the vegetable extracts**

The results of the qualitative analysis showed that each of the studied plant extract contains at least two classes of secondary metabolites such as alkaloids, anthocyanins, anthaquinones, flavonoids, phenols, saponins, tannins, sterols and triterpenes (Table 2). Only the extract from *A. hybridus* contains anthocyanins, while triterpenes were found both in this extract as well as that of *C. frutescens*. The extract from *C. frutescens* as well as those from *S. edule* and *M. esculenta* contained the highest number of classes of the studied secondary metabolites (five). Alkaloids and phenols were present in all vegetable extracts except that of *A. hybridus*.

**Antibacterial activity of the vegetable extracts**

The data summarized in Table 3 show the antibacterial activities of the tested extracts on a panel of twenty-nine Gram-negative bacteria. All extracts were active on at least twelve bacterial strains with MIC ≤ 1024 μg/mL. The extract of *C. frutescens* showed inhibitory activities against 16 (55.17%) of the 29 tested bacteria whilst that of *P. vulgaris* inhibited the growth of 12/29 (41.38%) pathogens (narrowest spectrum). None of these two extracts showed any antibacterial activity against *Pseudomonas* species, but were active against at least one bacterial strain of other studied genus. Extracts from *L. sativa*, *S. edule*, *C. pepo*...
and *S. nigrum* displayed the largest spectra of activity, their inhibitory effects being observed on all the 29 Gram-negative bacteria (100% of activity). The extracts from *A. hybridus*, *V. hymenolepis*, *L. sativa*, *L. carpensis* and *M. esculenta* also exhibited large spectrum of activity as they were active on 28/29 tested bacteria. The top eight active extracts, with large spectra of activity, showed MIC values generally ranging from 128 to 512 μg/ml. These MIC values were in some of the cases better than those of chloramphenicol (Table 3). This was the case with the extract from *V. hymenolepis* (MIC of 128 μg/ml) against *E. aerogenes* EA27. The extracts from *A. hybridus*, *S. edule* and *C. pepo* as well as those from *L. carpensis* and *M. esculenta* were more active than chloramphenicol on at least one of the tested MDR bacteria. The activity of chloramphenicol increased in the presence of PAβN in the majority of the tested bacteria (Table 3). The best activity was obtained with the extract from *A. hybridus* with the lowest MIC value of 128 μg/ml observed against 7/29 (25%) tested bacteria. The extracts from *P. vulgaris* and *C. frutescens* did not show any MBC value at up to 1024 μg/ml. Concerning the eight other vegetable extracts, the MBC results showed values equal to or below 1024 μg/ml in many cases. The extract from *C. pepo* leaves showed the best MBC spectrum with the values below to 1024 μg/ml recorded on 58,62% (17/29) of the studied microorganisms, best MBC spectrum with the values below to 1024 μg/ml (Table 4). The results of the phytochemical test on *P. vulgaris* are in accordance with some other reports [48,79]. *Phaseolus vulgaris* was found to inhibit also the growth of Gram-positive bacteria *B. subtilis* [49]. Amarowicz et al. [80] showed that the acetone extract of *P. vulgaris* contains tannins with good antimicrobial properties against *Listeria monocytogenes*. Therefore, the low antibacterial effects of this plant as obtained herein (generally MIC values at 1024 μg/ml) (Table 3) could be due to the multi-drug resistance ability of the studied bacteria.

Table 4 also shows that *M. esculenta* exhibited MBC values against all the strains of *E. aerogenes* and that, in general, the extracts showed values which were not 4-fold greater than the corresponding MICs.
### Table 1 Plant species used in this study and their reported effects

| Plant (family); and voucher number* | Traditional uses | Parts used traditionally | Bioactive or potentially bioactive components | Bioactivities of extracts and/or compounds |
|-------------------------------------|------------------|-------------------------|-----------------------------------------------|---------------------------------------------|
| **Amaranthus hybridus** Linn (Amarantaceae); 15630 HNC | intestinal bleeding, diarrhea and excessive menstruation [5,10] | Leaves, seeds | flavonoids, steroids, terpenoids, cardiac glycosides [5] alkaloid, saponin, tannins, phenols, hydrocyanic acid and phytic acid [11,12] | antimicrobial [5,13] |
| **Vernonia calva** (H.F.) Hook (Asteraceae); 27743 HNC | wounds [14], anticancer [15], fever, stomach ache, diarrhea, herna, spleen enlargement [16] | leaves | vernolepin [17,18], vernomenin [18], flavonoids (quercetin, apigenin, luteolin) [19] | cytotoxic [17], spasmylic, anti-aggregating and de-aggregating activities, 2 antitumor activity, antimicrobial [20], insecticide [21], antifilarial [22] |
| **Lactuca sativa** Linn; (Asteraceae); 25624/SRF.Cam | analgesic, conjunctivitis, tired eyes, Insomnia, sedative [23] insomnia, anxiety, neuralgia, dry coughs, rheumatic pain [24] stimulate digestion, enhance appetite and relieve inflammation [25] | leaves | phenolic acids, triterpenoids, saponins, phytol [23], carotenoids [26], flavonoids including kaempferol [19] Lettucenin-A guaianolide sesquiterpenelactones conjugates, lactucin, deoxy lactucin and lactucopicrin [27] | antimicrobial [28], antifungal, antibacterial [29], antitumor [30] antioxidating, analgesic, and anti-inflammatory [23] depressant [31] sedative, hypnotic, analgesic and anticonvulsant [32] hypoglycaemic [33] antioxidant I [34,35], and anxiolytic |
| **Lactuca capensis** Thumb (Asteraceae); 27743 HNC | anti spasmodic, digestive, diuretic, hypnotic, narcotic and sedative properties. Treatment of insomnia, anxiety, neuralgia, hyperactivity in children, dry coughs, whooping cough, rheumatic pain, chronic joint pains [30] | leaves | luctacarium, sesquiterpene lactone [37] | |
| **Sechium edule** (Jacq) SW (Cucurbitaceae); 42459/HNC | urine retention, kidney diseases, arteriosclerosis, hypertension [38] | leaves | C-glycosyl and O-glycosyl flavonoids, saponins and saponins [38] | diuretic [9], free radical scavenger and antioxidant [40], antibacterial [41], antihypertensive [42], hepatoprotective activity of ethanolic extract and its different [43] |
| **Manihot esculenta** Crantz (Euphorbiaceae); 57650/HNC | hypertension, headache and pain, irritable bowel syndrome, fever, headache, aches and pains [44] | leaves | 3-rutinosides of kaempferol and quercetin; the cyanogenic glycosides, lotaustralin and linamarin, from the fresh leaves of cassava [45] | antihelminthic activity of crude extracts antibacterial [46] |
| **Phaseolus vulgaris** Linn (Fabaceae); 42587/HNC | osteoporosis prevention, diuretic, eczema, antihyperglycemic [47] | leaves | ascorbic acid, phenol, alkaloids, sterols, saponins (aqueous extract), carotenoids like lutein, β-carotene, violanthin and neoxanthin, flavonoids [48] including quercetin, kaempherol, catechins, epicatechins and procyanidins | antioxidatant [48], antibacterial [49] |
| **Cucurbita pepo** Linn (cucurbitaceae); 15630 HNC | intestinal infections and kidney problems (seeds), minor injuries (flowers), anthelmintic, hypertension, erysipelas, enteritis, dyspepsia, stomach disorders, liver disorders like jaundice [50] | leaves | saponin, tannin, quinone, coumarins, flavonoids, steroid, terpenes, lignin, alkaloids, protein and sugar Curcicin [52] anthocyanin, phenols like syringic acid [52], phytin, lecithin, cucurbitane and hexacurcurbutane L-2-O-β-glucopyranoside, Curcicin [52], flavonoids, Vitamins B, C, and E, β-sitostérol | antihypertensive, anti-oxidative activities, Arthritis, reduce the symptoms of BPH [52,53]. High Cholesterol, anti-parasitic activity in vi-vitro [54], alleviates the detrimental effects associated with protein malnutrition [55], antiparasitic [56], nephron and hepato-protective, vermifuge, inhibitor of prostaglandin biosynthesis [57], antiparasitic, protects gastric mucosal [50] |
| **Solanum nigrum** Linn (Solanaceae); 43000 HNC | pneumonia aching teeth, stomach ache, tonsilitis, tonic, cold worms [14], pain, inflammation and fever, tumor, antioxidant, anti-inflammatory, hepatoprotective, diuretic, anti-pyretic [58] | leaves | kaempferol [19,59] terpenoids and condensed tannin [60], quercetin, flavonoids [19], polysaccharides, polyphenolic compounds including galic acid, catechin, caffeic acid, rutin and naringenin [58] | anti-inflammatory, antioxidant, anthelmintic activity [60] antiinocceptive, antipyretic, antitumor, antilucreogenic, cancer chemopreventive, hepatoprotective, and immunomodulatory effects [61] Mosquito larvicidal [62], antibacterial [63] |
of *M. esculenta* against MDR strains of *P. aeruginosa*, *E. coli*, *E. cloacae*, *K. pneumoniae*, *P. stuartii* and *E. aerogenes*. The activity of *Amaranthus hybridus* was reported against *E. coli*, *S. typhi*, *K. pneumoniae* and *P. aeruginosa* with MICs ranged between 200 and 755 mg/ml [5]. The ethyl acetate extract exhibited activity against *S. aureus* and *B. subtilis* whilst the ethanol extract was found effective against *E. coli* [13].

The high MIC values observed with chloramphenicol can be explained only if we take into account the non-specific resistance mechanism: active efflux of the toxic compound by pumps belonging to the small multidrug resistance (SMR) proteins family [4]. The fact that the efflux pump inhibitor (PAβN) enhances the chloramphenicol antibacterial properties is a clear indication that the tested strains express an active efflux system and that this system is responsible for resistance of the tested bacteria to chloramphenicol. The wide substrate specificity of these pumps, as well as their widespread among bacterial species make us believe that these efflux pumps are also responsible for the extrusion of various active compounds from the plant extract out of bacteria cells, therefore preventing their inhibitory effects. Therefore, the activities of the vegetable as observed herein against MDR strains (with MIC comprised between 128 and 1024 μg/mL) could be considered important, especially when considering the fact that we are dealing with edible plants. Apart for the extracts of *P. vulgaris* and *C. frutescens* which did not show any MBC below 1024 μg/mL, other values further confirmed the bactericidal effect of the 8 remaining extracts as they were generally less than 4-fold greater than corresponding MIC values [82,83].

**Conclusions**

The overall results of the present investigation confirmed the traditional uses of the studied vegetables in the treatment of bacterial infections. This study also provide baseline information for the possible use of the methanol extracts of the tested plant samples in the control of infectious diseases involving Gram-negative MDR bacteria. The arising question is of course which are the active compounds responsible for these effects. Our research group is currently focusing on the characterization of these plants extracts in terms of chemical composition and synergistic effects.

#### Table 1 Plant species used in this study and their reported effects (Continued)

| Scientific names | Part used | Yield (%) | alkaloids | phenols | tannins | terpènes | stéroids | flavonoids | anthraquinones | anthocyanins | saponins |
|------------------|-----------|-----------|-----------|----------|---------|----------|----------|------------|----------------|-------------|----------|
| *Capsicum frutescens* L. (Solanaceae); 10737/SRFCa | leaves | 7.9 | - | - | - | + | - | + | - | + |
| *Vernonia hymenolepis* | leaves | 9.40 | + | + | - | - | + | - | - | - |
| *Lactuca sativa* | leaves | 7.14 | + | + | - | - | + | - | - | - |
| *Lactuca capensis* | leaves | 7.14 | + | + | + | - | + | - | - | - |
| *Sechium edule* | leaves | 3.76 | + | + | - | + | + | - | - | + |
| *Manihot esculenta* | leaves | 0.46 | + | + | + | + | + | + | - | + |
| *Phaseolus vulgaris* | clove | 17.81 | + | + | - | - | + | + | - | - |
| *Cucurbita pepo* | leaves | 12.68 | + | + | - | - | + | + | - | - |
| *Solanum nigrum* | leaves | 11.84 | + | + | - | - | + | + | - | - |
| *Capsicum frutescens* | fruits | 16.24 | + | + | - | - | + | + | - | - |

*(HNC): Cameroon National Herbarium; (SRFC): Société des Réserves Forestières du Cameroun.

#### Table 2 Extraction yields and phytochemical composition of the plant extracts

| Scientific names | Part used | Yield (%) | alkaloids | phenols | tannins | terpènes | stéroids | flavonoids | anthraquinones | anthocyanins | saponins |
|------------------|-----------|-----------|-----------|----------|---------|----------|----------|------------|----------------|-------------|----------|
| *Amaranthus hybridus* | leaves | 7.9 | - | - | - | + | - | + | - | + | - |
| *Vernonia hymenolepis* | leaves | 9.40 | + | + | - | - | + | - | - | - | - |
| *Lactuca sativa* | leaves | 7.14 | + | + | - | - | + | - | - | - | - |
| *Lactuca capensis* | leaves | 7.14 | + | + | + | - | + | - | - | - | - |
| *Sechium edule* | leaves | 3.76 | + | + | - | + | + | - | - | + | - |
| *Manihot esculenta* | leaves | 0.46 | + | + | + | + | + | + | - | + | - |
| *Phaseolus vulgaris* | clove | 17.81 | + | + | - | - | + | + | - | - | - |
| *Cucurbita pepo* | leaves | 12.68 | + | + | - | - | + | + | - | - | - |
| *Solanum nigrum* | leaves | 11.84 | + | + | - | - | + | + | - | - | - |
| *Capsicum frutescens* | fruits | 16.24 | + | + | - | - | + | + | - | - | - |

*(+): Present; (−): Absent; *The yield was calculated as the ratio of the mass of the obtained methanol extract/mass of the plant powder.
Table 3 Susceptibility of bacteria to plant extracts - MICs of methanol extracts vs chloramphenicol

| Bacteria strains | MIC (μg/ml) of the plant extracts |
|------------------|----------------------------------|
|                  | A. hybridus | V. hymenolepis | L. sativa | L. capensis | S. edule | M. esculenta | P. vulgaris | C. pepo | S. nigrum | C. frutescens | Chloramphenicol² |
|                  |            |                |           |            |         |              |            |        |          |               |                  |
| E. coli          |            |                |           |            |         |              |            |        |          |               |                  |
| ATCC8739        | 256        | 1024           | 512       | 512       | 256     | 256          | 1024       | 512    | 512       | 512          | 4                 |
| ATCC10536       | 128        | 256            | 128       | 256       | 128     | -            | 256        | 128    | -          | 4             |                  |
| W3110           | 256        | 512            | 256       | 256       | 512     | -            | 128        | 256    | -          | 8 (< 2)       |                  |
| MC4100          | 512        | 1024           | 512       | 1024      | 256     | 512          | 1024       | 256    | 512       | 1024         | 16 (< 2)         |
| AG100A          | 256        | 512            | 512       | 512       | 512     | 512          | -          | 512    | 512       | 1024         | < 2 (< 2)        |
| AG100Atet       | 256        | 512            | 512       | 512       | 512     | -            | 512        | 512    | 1024      | 64 (< 2)      |                  |
| AG1012          | 1024       | 128            | 1024      | 512       | 512     | 128          | -          | -      | 256       | 512          | 64 (< 2)         |
| AG100           | 128        | 1024           | 128       | 512       | 512     | 512          | -          | 512    | 256       | -             | 8 (< 2)          |
| E. aerogenes     |            |                |           |            |         |              |            |        |          |               |                  |
| ATCC13048       | 128        | 1024           | 256       | 256       | 256     | 256          | 1024       | 256    | 256       | -            | 8                 |
| EA294           | 512        | 512            | 512       | 512       | 512     | 1024         | -          | 512    | 512       | 1024         | 16                |
| CM64            | 128        | 128            | 256       | 256       | 128     | 256          | 1024       | 512    | 512       | 512          | 256 (8)         |
| EA3             | 256        | 256            | 128       | 128       | 256     | 128          | 1024       | 128    | 128       | -             | 256 (128)       |
| EA298           | 256        | 512            | 256       | 256       | 512     | 512          | 1024       | 128    | 256       | 512          | 1024             |
| EA27            | 512        | 512            | 256       | 512       | 512     | -            | 512        | 512    | 256       | 512          | ≥ 256 (< 2)     |
| EA289           | -          | 512            | 1024      | 256       | 512     | 512          | 512        | 1024   | 512       | 256          | ≥ 256 (64)       |
| K. pneumoniae   |            |                |           |            |         |              |            |        |          |               |                  |
| ATCC11296       | 256        | 512            | 256       | 512       | 512     | 512          | 512        | 512    | 256       | -            | 8                 |
| KP55            | 256        | 512            | 256       | 512       | 256     | 512          | 512        | 256    | 256       | 256          | 32 (4)          |
| KP63            | 256        | 256            | 256       | 256       | 256     | 256          | 512        | 1024   | 128       | -             | 64 (< 2)         |
| K2              | 512        | -              | 512       | 512       | 1024    | 512          | -          | 1024   | 512       | 1024         | 32 (< 2)         |
| K24             | 512        | 1024           | 512       | 512       | 512     | -            | 1024       | 512    | 512       | 1024         | 16 (< 2)         |
| P. aeruginosa   |            |                |           |            |         |              |            |        |          |               |                  |
| PA01            | 256        | 512            | 512       | 256       | 256     | 512          | -          | 256    | 512       | -             | 16                |
| PA124           | 512        | 1024           | 512       | 512       | 512     | 512          | -          | 512    | 512       | -             | 32 (< 2)         |
| P. stuartii     |            |                |           |            |         |              |            |        |          |               |                  |
| ATCC29916       | 128        | 128            | 256       | 1024      | 128     | 1024         | -          | 1024   | 256       | -             | 16                |
| NAE16           | 128        | 512            | 256       | 256       | 256     | 256          | 1024       | 512    | 256       | -             | 64 (8)          |
| PS2636          | 512        | 512            | 256       | 256       | 256     | 256          | -          | 256    | 256       | 512          | 32                |
| PS299645        | 512        | 1024           | 512       | 512       | 512     | 512          | -          | 512    | 1024      | -             | 32 (< 2)         |
| E. cloacae      |            |                |           |            |         |              |            |        |          |               |                  |
| BM47            | 128        | 256            | 512       | 1024      | 256     | 1024         | -          | 128    | 512       | -             | ≥ 256 (< 2)     |
| ECC69           | 256        | 512            | 512       | 256       | 256     | 512          | -          | 256    | 512       | -             | ≥ 256 (16)       |
| BM67            | 256        | 512            | 512       | 256       | 256     | 512          | 1024       | 128    | 512       | 1024         | 128 (32)         |

The results are shown as average values from three separate experiments.

(−) MIC > 1024 μg/ml.

¹ - chloramphenicol was used as a reference antibiotic. MIC was measured in absence and presence of PAßN (in brackets).

² chloramphenicol was used as a reference antibiotic. MIC was measured in absence and presence of PAßN (in brackets).
| Bacteria strains | A. hybridus | V. hymenolepis | L. sativa | L. capensis | S. edule | M. esculenta | Green bean (P. vulgaris) | C. pepo | S. nigrum | C. frutescens | Chloramphenicol |
|------------------|-------------|----------------|---------|-----------|--------|------------|---------------------|--------|--------|-------------|----------------|
| *E. coli*        |             |                |         |           |        |            |                     |        |        |             |                |
| ATCC8739         | -           | -              | -       | -         | 1024   | 1024       | -                   | 512    | -      | -           | 64              |
| ATCC10536        | 1024        | -              | -       | -         | -      | -          | 1024                | -      | -      | 1024        | 128             |
| W3110            | 1024        | 512            | 256     | -         | 512    | -          | 512                 | -      | -      | -           | -               |
| MC4100           | 1024        | -              | -       | -         | -      | -          | -                   | -      | -      | -           | -               |
| AG100A           | -           | 1024           | 512     | -         | -      | -          | -                   | -      | -      | -           | -               |
| AG100A\text{tet} | -           | -              | 512     | 1024      | -      | -          | -                   | -      | -      | -           | -               |
| AG102            | -           | -              | 512     | 1024      | -      | -          | -                   | -      | -      | -           | -               |
| AG100            | 256         | 1024           | -       | 1024      | -      | 512        | 1024                | -      | -      | -           | -               |
| *E. aerogenes*    |             |                |         |           |        |            |                     |        |        |             | 512             |
| ATCC13048        |             |                |         |           |        |            |                     | 1024   | 1024   | -           | 128             |
| EA294            | -           | -              | -       | 1024      | -      | -          | -                   | -      | -      | -           | 32              |
| CM64             | 512         | -              | 512     | 512       | 512    | -          | -                   | -      | -      | -           | -               |
| EA3              | 1024        | 512            | 1024    | 1024      | -      | 512        | 1024                | -      | 1024   | -           | -               |
| EA298            | 512         | 1024           | 1024    | 1024      | 256    | -          | 256                 | 512    | -      | -           | -               |
| EA27             | -           | -              | -       | -         | 512    | -          | -                   | -      | -      | -           | -               |
| EA289            | -           | 1024           | -       | 512       | 1024   | 1024      | 512                 | -      | -      | -           | -               |
| *K. pneumoniae*   |             |                |         |           |        |            |                     |        |        |             |                |
| ATCC11296        | -           | -              | -       | 1024      | -      | -          | 256                 | 1024   | -      | 64          |                |
| KP55             | 1024        | -              | -       | 1024      | 1024   | -          | 1024                | 512    | -      | 128         |                |
| KP63             | 512         | 512            | -       | -         | -      | -          | -                   | 512    | 1024   | -           | -               |
| K2               | 1024        | -              | 1024    | -         | -      | -          | -                   | -      | -      | 256         |                |
| K24              | -           | -              | -       | 1024      | -      | -          | -                   | 512    | -      | 512         |                |
| *P. aeruginosa*  |             |                |         |           |        |            |                     |        |        |             |                |
| PA01             | -           | -              | -       | -         | -      | -          | -                   | -      | -      | -           | 256             |
| PA124            | -           | 1024           | 1024    | -         | -      | -          | 1024                | 512    | -      | -           | -               |
| *P. stuartii*    |             |                |         |           |        |            |                     |        |        |             |                |
| ATCC29916        | -           | 256            | -       | 1024      | 1024   | 1024       | -                   | 1024   | 512    | -           | 128             |
| NAE16            | -           | -              | 512     | 1024      | -      | -          | -                   | 1024   | -      | -           | 256             |
| PS2636           | 512         | 1024           | 1024    | 1024      | -      | 512        | -                   | 512    | -      | -           | -               |
| PS299645         | -           | -              | -       | -         | -      | -          | -                   | -      | -      | -           | -               |
| *E. cloacae*     |             |                |         |           |        |            |                     |        |        |             |                |
| BM47             | -           | 1024           | 512     | -         | -      | -          | -                   | 1024   | -      | -           | -               |
| ECCI69           | 1024        | 512            | -       | 1024      | 512    | 1024       | -                   | 1024   | 512    | -           | -               |
| BM67             | 512         | 1024           | 1024    | 1024      | 1024   | 1024       | -                   | 1024   | -      | -           | -               |

The results are shown as average values from three separate experiments.  
(−) MBC > 1024 μg/ml.  
(−) chloramphenicol was used as a reference antibiotic.
Additional file

**Additional file 1: Table S1.** Bacterial strains and features.

Competing interest
The authors declare that they have no competing interest.

Authors' contributions
JAKN, MM, STL and MS carried out the study; VK designed the experiments. JAKN, MM and VK wrote the manuscript; VK and JRK supervised the work; VK provided the bacterial strains; all authors read and approved the final manuscript.

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