IMP-1 encoded by a novel Tn402-like class 1 integron in clinical Achromobacter xylosoxidans, China

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Achromobacter xylosoxidans strain A22732 is isolated from a pneumonia patient in China and produces carbapenemases OXA-114e and IMP-1, which are encoded by chromosome and plasmid, respectively, and confer resistance to multiple β-lactam antibiotics including carbapenems. The blaIMP-1 gene together with aacA7 and orfE is captured by a novel Tn402-like class 1 integron in a conjugative IncP-1ß plasmid. In addition to the intrinsic integron promoter PcW, there is still a blaIMP-1 gene cassette-specific promoter. This is the first report of carbapenemase-encoding IncP-1ß plasmid in clinical bacterial isolate.

Results and Discussion
Achromobacter xylosoxidans is a Gram-negative, motile bacterium from the Achromobacter genus of the Alcaligenaceae family, and frequently found in water environments and can also cause opportunistic infections especially in immunocompromised patients.

A. xylosoxidans expresses a chromosomally encoded intrinsic carbapenemase OXA-114 (Ambler class D) in a constitutive manner1 with at least 22 variants OXA-114a to OXA-114v (http://www.ncbi.nlm.nih.gov/nuccore/, last accessed May 9, 2014), and PCR detection of blaOXA-114 genes has been established for species identification of this bacterium2.

The IMP-type metallo-carbapenemases (class B) are composed of at least 48 variants IMP-1 to IMP-48 (http://www.lahey.org/studies/, last accessed May 9, 2014), and they are found in many clinical Gram-negative bacteria including Pseudomonas spp., Acinetobacter spp., and members of the Enterobacteriaceae family3. At least three IMP variants, namely IMP-1 and IMP-194, and IMP-105, have been detected in A. xylosoxidans isolates from Japan; all of these blaIMP-1 genes are carried on plasmid-borne class 1 integrons, but the complete nucleotide sequences of these IMP-encoding plasmids are not determined.

The present study describes the complete nucleotide sequence of a conjugative IncP-1ß plasmid from a clinical A. xylosoxidans isolate from China, carrying a novel Tn402-like class 1 integron that includes the blaIMP-1 gene cassette.

Achromobacter xylosoxidans strain A22732 harboring a conjugative IMP-encoding plasmid. In September 2010, an 86-year-old male was admitted to our hospital and diagnosed to have pneumonia, and sputum specimens were sampled on the same day. The next day, bacterial growth was observed after cultivation of sputum on Mueller-Hinton agar, and the bacterial isolate designated A22732 was identified as A. xylosoxidans by VITEK 2, Bruker MALDI Biotyper, and 16s rRNA gene sequencing. The antimicrobial susceptibility test using VITEK 2 indicated A22732 was resistant to multiple β-lactam antibiotics including imipenem and meropenem but remained susceptible to fluoroquinolones, and the patient then received intravenous administration with moxifloxacin hydrochloride, and he was cured after ten days of antimicrobial treatment.

Positive PCR amplification of two carbapenemase genes blaIMP and blaOXA-114 was observed for strain A22732, which was further validated by PCR amplicon sequencing, but all the remaining known carbapenemase and extended spectrum β-lactamase (ESBL) genes tested gave negative PCR results. The whole gene amplification/sequencing indicated the presence of intact blaOXA-114e gene in A22732. A blaIMP-positive and blaOXA-114e-negative strain was isolated from the sputum of the patient. This is the first report of carbapenemase-encoding IncP-1ß plasmid in clinical bacterial isolate.
negative E. coli transconjugant, designated A22732-IMP-EC600, was obtained by conjugal transfer, indicating that A22732 harbored a conjugal IMP-encoding plasmid, which was designated pA22732-IMP. As determined by a modified CarbaNP test\(^a\), strain 22732-IMP-EC600 had class B carbapenemase activity, while A22732 probably expressed class B/D carbapenemases (Fig S1), being consistent with the above PCR/sequencing results.

The minimum inhibitory concentration (MIC) values (Table 1) were determined for A22732, 22732-IMP-EC600, and EC600. A22732 and 22732-IMP-EC600 show almost identical drug resistance profiles. These two strains are highly resistant to penicillins, aztreonam, and cephalosporins tested, but they remain susceptible to ampicillin, sulfamethoxazole, and ciprofloxacin. Both A22732 and 22732-IMP-EC600 are resistant to aminoglycosides and 3-\(\beta\)-lactams tested, but they remain susceptible to sulfamethoxazole/sulfamethoxypyridazine.

22732-IMP-EC600 had class B carbapenemase activity, while A22732 and 22732-IMP-EC600 are resistant to ampicillin, carbenicillin, and cephalosporins tested, but they remain susceptible to sulfonamides.

The DNA sequence of 49,804-bp with a total of 60 complete genes belongs to IncP-1\(\beta\) plasmid pA22732-IMP, which harbors only a single one IMP-1. The drug resistance genes encoding IMP-1 and OXA-114 were also detected. Additionally, pA22732-IMP encodes an IMP-1 carbapenemase and an IMP-1 like carbapenemase. 22732-IMP-EC600 produces those (4 and 2, respectively) against 22732-IMP-EC600, which is resistant to the corresponding region of a set of IncP-1\(\beta\) broad-host-range plasmids. By contrast, only Tn501-like element rather than Tn402-like integron is found in the corresponding region of pAKD31 (Fig 2).

### Tn501-like elements

Tn501-like transposon insertions, which often harbor the mer locus conferring mercuric chloride resistance, are frequently found in IncP-1\(\beta\) plasmids from agricultural soils\(^\text{9,10,12}\). The ancestral Tn501-like mer locus (Fig 3) contains the Tn501 transposition genes tspA (transposase) and tnpR (resolvase), the mercury-resistance genes merR (repressor of mer locus), merT (integral membrane protein for mercuric transport), merP (periplasmic mercury ion-binding protein), merA (mercuric reductase), merD (co-regulator protein), merE (integral membrane protein for mercuric transport) and orf2 (EAR-domain-containing protein), and the 38-bp terminal inverted repeats (left terminal inverted repeat IRI, and right terminal repeat RIR)\(^b\).

Tn501-like loci can be identified in all the above five plasmids (Fig 3). The Tn501-like element of pB10 is flanked by a 5-bp target site (direct repeats of TGGCCT), but those of all the other four plasmids leave no trace of insertion. pB10 harbors an greatly extended Tn501-like mer locus, because tspA is interrupted by IS1071 (which is further interrupted by Tn1271-like tetracycline-resistance locus) plus a Tn5393-like streptomycin resistance locus, thereby leading to partial deletion of tspA. Loss of IRI and tnpAR as well as 3’-terminus deletion of orf2, because of insertion of IS1071 plus IS21 into orf2, is observed for the Tn501-like mer locus of pAKD31. The Tn501-like mer locus of pA22732-IMP has undergone loss of IRI, tnpAR, and orf2 as well as an inversion event of the whole locus.

Both R751 and pB8 carry a ‘cryptic’ Tn501-like element, which is composed of IRI, orf1, orf2, a merR remnant and IRI in the absence of all the other features identified for the ancestral Tn501-like mer locus. In addition, R751 has acquired two copies of IS4321 (IS4321L and IS4321R), which flank the merR remnant and orf2, respectively. By contrast, evolution of pB8 involves insertion of a Tn501-like quaternary ammonium compound resistance (qacF) locus into orf2, thereby disrupting this gene.

### Tn402-like integrons

Tn402 is bound by 25-bp IRi and IRi (see below), and acts as the primary carrier element of class 1 integrons\(^\text{11-13}\). Tn402-like class 1 integrons are identified in pB8, pA22732-IMP, R751 and pB10, but not pAKD31 (Fig 4a). The pB8 integron is a typical class 1 integron integron that, from 5’ to 3’ side, harbors 5’-conserved segment (CS)-specific integrase gene intI1, resistance gene cassettes bla\(_{\text{OXA-2}}\) (class D \(\beta\)-lactamase OXA-2) and aadA4 (spectinomycin/ streptomycin resistance), 3’-CS-specific gene cluster qacEAl-1-sulf- orf5 and Tn402 transposition proteins intAB\(^c\). The pA22732-IMP integron contains intI1, aacA7 (gentamicin/amikacin resistance), orfE, blA\(_{\text{IMP-1}}\), and truncated intA, being atypical due to lack of 3’-CS. Notably, 3’-CS-lacking integrons have been already observed in

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Table 1 | MIC values and antimicrobial susceptibility

| Antibiotics | A22732 | 22732-IMP-EC600 | EC600 |
|-------------|--------|----------------|------|
| Ampicillin  | >32/R  | >32/R          | 16/1 |
| Ampicillin/sulbactam | >32/R  | >32/R          | ≤2/S |
| Aztreonam  | >64/R  | ≤1/S           | ≤1/S |
| Cefazolin  | >64/R  | >64/R          | ≤4/S |
| Cefuroxime sodium | >64/R  | >64/R          | 16/1 |
| Cefuroxime axetil | >64/R  | >64/R          | 16/1 |
| Cefotaxone | >64/R  | >64/R          | >4/S |
| Cefotaxime | >64/R  | >64/R          | >4/S |
| Cefoxazidine | >64/R  | >64/R          | >4/S |
| Cefepime   | >64/R  | >64/R          | >4/S |
| Imipenem   | >16/R  | >16/R          | ≤1/S |
| Meropenem  | >16/R  | 2/R            | ≤0.25/S |
| Ciprofloxacin | 1/S    | ≤0.25/S        | ≤0.25/S |
| Levofloxacin | 2/S    | 0.5/2/S        | ≤0.25/S |
| Macrolin   | 256/R  | ≤16/S          | 16/S |
| Amikacin   | >64/R  | ≤2/S           | ≤2/S |
| Gentamicin | >16/R  | ≤1/S           | ≤1/S |
| Tobramycin | >16/R  | 4/S            | ≤1/S |
| Trimethoprim/sulfamethoxazole | ≤20/S  | ≤20/S          | ≤20/S |

S: sensitive; R: resistant; I: intermediate.
Figure 1 | Schematic maps of plasmid pA22732-IMP. Genes are denoted by arrows and colored based on gene function classification. The innermost circle presents GC-Skew \((\frac{G-C}{G+C})\) with a window size of 500-bp and a step size of 20-bp. The blue circle presents GC content. Shown also are backbone (orange) and accessory module (yellow) regions of pA22732-IMP.

Figure 2 | Linear comparisons of sequenced plasmids. Genes are denoted by arrows and colored based on gene function classification.
Figure 3 | Linear comparisons of Tn501-like elements. Genes are denoted by arrows and colored based on gene function classification.

Figure 4 | Schematic representation of Tn402-like integrons and flanking regions. Genes are denoted by arrows and colored based on gene function classification.
Figure 5 | Organization and expression of integron gene cassettes. a) Operon structure. Boxed arrows stand for length/direction of indicated genes. The two broken-line arrows represent primary RNA transcripts transcribed for the aacA7-orfE-blaIMP-1 operon and the blaoIMP-1 gene, respectively. Line with filled circles at both termini indicates location of primer pair plus expected PCR amplicon. b) PCR and RT-PCR. cDNAs generated from total RNA of strain A22732, and genomic DNA of A22732 were used as templates for RT-PCR and PCR, respectively. c) Primer extension. Primer extension assay of the RNA transcript of aacA7 or blaoIMP-1 was done for A22732 cultured with addition of increasing amounts of imipenem. Lanes C, T, A and G represent Sanger sequencing reactions. The transcription start of aacA7 or blaoIMP-1 is indicated by the arrow with nucleotide T or G, respectively, and the minus number under arrow indicate the nucleotide position upstream of the aacA7 or blaoIMP-1 start codon. Representative data from at least two independent biological replicates are shown.

many cases. The R751 integron contains dhoFIlc (trimethoprim resistance), orfD, truncated qacE, and complete Tn402 transposition module tniABQC. The pB10 integron harbors intIl, blaoXAc2, orfE, and qacEA1-sul1-orfS.

At least three key steps (Fig S2) are involved in evolution of Tn402-like class 1 integrons: step I, insertion of ancestor class 1 integron (lack of 3'-CS) into Tn402 (harboring complete tniABQC transposition module) to generate a hybrid structure, combining the ability of integron to capture environmental gene cassettes to the mobility of Tn402 into plasmids and other genetic platforms, which might occur prior to or concomitant with antibiotic era including capture of qacE (quaternary ammonium compound resistance); step II, capture of sulI (sulfonamide resistance) and orf5, and then formation of 3'-CS due to deletion events between qacE and sulI; step III, deletion events within tniABQR, making Tn402 transposition incompetent.

The pB8 and pB10 integrons, each of which contain 5'-CS, drug resistance gene cassettes, 3'-CS, and completely or partially truncated tni module, appear to undergo all the above three steps of evolution. By contrast, the evolution step II (formation of 3'-CS) is mostly likely omitted for the pA22732-IMP integron, while the R751 integron might represent a primitive Tn402-like integron due to absence of evolution steps II and III (truncation of tni module).

The Tn402-like integron of pA22732-IMP is inserted into the traC-parA integric region, leaving parA and its downstream gene upf31.0 intact. The parA upstream or around region represents a hot spot for Tn402 targeting, most likely due to it contains the multimer resolution site II for recognition by Tn402 transposase. Interestingly, the insertion of Tn402-like integrons into the hotspot target region leads to further deletion of parA from pB8 and R751, and that of parA/upf31.0 from pB10.

The pA22732-IMP, R751 and pB8 integrons contains IRi and IRt of Tn402, but only IRt rather than IRi is identified for pB10. The lack of IRt in pB10 might due to the above-mentioned deletion removing of parA/upf31.0. The 5-bp target site (direct repeats of AGCAT) is still intact to flank the pA22732-IMP integron, but all the other three integrons do not leave traces of insertion.

Integrase IntIl recognizes two different types of recombination site attI (integron attachment site) and attC (recognition site for integrase), and it catalyzes integration or excision of gene cassettes through site-specific recombination commonly between one attI site and one or more attC sites. One attI site and three attC sites, upstream of aacA7, orfE, blaoIMP-1 and tniA, are indentified in the pA22732-IMP integron (Fig 4a). These sites are long inverted-repeat-containing sequences of variable length and sequence, and each inverted repeat begins with a core sequence RYYYAAC and ends with an inverted core sequence GTTRRRY as described previously, which would form imperfect cruciform structures and be required for capture of aacA7, orfE, and blaoIMP-1.

Expression of integron gene cassettes. The pA22732-IMP integron gene cassettes aacA7, orfE and blaoIMP-1, but not intIl and tniA, are organized in the same transcriptional direction (Fig 5a). PCR generates an amplicon ranging from 5'-untranslated region (5'-UTR) of aacA7 to 5'-terminal of the blaoIMP-1 coding region, when using A22732 genomic DNA as template (Fig 5a). The positive RT-PCR amplification with the same primer pair, using cDNA sample generated from A22732 total RNA as template (Fig 5b), indicates that aacA7, orfE, and blaoIMP-1 are transcribed into a single RNA transcript and thereby, these three genes constitutes a single operon aacA7-orfE-blaIMP-1 (Fig 5a).
Integrins act as natural gene expression platforms due to the presence of an intrinsic promoter (Pc) that is recognized by RNA polymerase to drive transcription of inserted gene cassettes that generally do not have their own promoters. At least eight distinct types of Pc promoter, PcS (strong), TTGACA-N₁₇-TAAACT, PcW (weak), TTGATA-N₁₇-TAAACT, PcH1 (hybrid 1, TTGACA-N₁₇-TAAACT), PcH2 (Hybrid 2, TTGACA-N₁₇-TAAACT), PcSS (super-strong, TTGATA-N₁₇-TAAACT), PcIn42 (TTGACA-N₁₇-TAAACT), PcIn116 (TTGACA-N₁₇-TAAACT), and PcPOU (TTGACA-N₁₇-TAAACT) have been described for class 1 integrons. As detected by primer extension (Fig 5c), a transcription start site (nucleotide C) is located at 227-bp upstream of aacA7 start codon (i.e. a 227-bp 5'-UTR of aacA7), validating presence of the PcW promoter to drive aacA7-orfE-blaIMP-1 transcription. The PcW promoter can be found for all the pA22732-IMP, pB8, R751, and pB8 integrons.

Remarkably, the primer extension assay detects another transcription start site (nucleotide G) which is located at 155-bp upstream of blaIMP-1 start codon. This assay discloses presence of an internal promoter (TTGCAA-N₁₇-TATCAT) driving transcription of the blaIMP-1 gene cassette (Fig 5c). Being very rare, the internal gene cassette-specific promoter is an extra element in evolution of anti-microbial resistance phenotype and act independent of Pc promoter27.

In addition, the primer extension assay shows that addition of increasing amounts of imipenem during bacterial cultivation has no effect on promoter activity of either aacA7-orfE-blaIMP-1 or blaIMP-1 (Fig 5c), validating constitutive expression of the aacA7-orfE-blaIMP-1 operon and the blaIMP-1 gene cassette. Notably, constitutive transcription of integron gene cassettes has been suggested previously27,28,29.

Concluding remarks. We present the first complete sequence of IMP-encoding plasmid from A. xylosoxidans, and this is also the first report of identification of a carbapenemase-encoding IncP-1β plasmid from a clinical bacterial isolate. The detected blaIMP-1 gene is captured by a novel class 1 integron with a novel gene cassette array in the IncP-1β plasmid P22732-IMP. The class 1 integron is embedded in a Tn402-like transposon and inserted into pA22732-IMP by transposition. Most of the characterized IncP-1β plasmids are isolated from bacteria in agricultural soils or waters and frequently associated with mercury resistance9,10,11,12. Only a few of them, e.g. pA22732-IMP and R75, are of clinical origins and harbor an array structure of multiple resistance gene cassettes. The IncP-1β plasmids thus could represent important vehicles for spreading clinically relevant resistance determinants across a number of bacterial species. The transfer of blaIMP to A. xylosoxidans, which already carried blaOXA-114 intrinsically, would lead to more severe drug resistance, making high difficulty in timely choosing sensitive antibiotics for treatment. The IMP-producing A. xylosoxidans should be taken seriously as the surveillance target especially in East Asia countries such as China and Japan.

Methods

Bacteria isolation and identification. Fresh sputum specimens were sampled from the indicated patient and inoculated onto Mueller-Hinton agar for bacterial isolation. The use of human specimens and all related experimental protocols were approved by the Committee on Human Research of Chinese People’s Liberation Army General Hospital and carried out in accordance with the approved guidelines, and moreover the informed consent was obtained from the indicated patient. Single colony of each bacterial strain tested was subjective for species identification by VITEK 2 (Biomerieux), Bruker MALDI Biotyper, and 16s rRNA gene sequencing. For different cell cultures (lanes) in a single experiment, equal amounts of total RNA were used as starting materials. The corresponding end-labeled primer was also used annealed with total RNA sample of strain A22732 for primer extension assay as described previously30. For different cell cultures (lanes) in a single experiment, equal amounts of total RNA were used as starting materials. The corresponding end-labeled primer was also used annealed with total RNA sample of strain A22732 for primer extension assay as described previously31. For different cell cultures (lanes) in a single experiment, equal amounts of total RNA were used as starting materials. The corresponding end-labeled primer was also used annealed with total RNA sample of strain A22732 for primer extension assay as described previously31. For different cell cultures (lanes) in a single experiment, equal amounts of total RNA were used as starting materials. The corresponding end-labeled primer was also used annealed with total RNA sample of strain A22732 for primer extension assay as described previously31.

PCR detection of bla genes. All known carbapenemase and ESBL genes as listed in Table S1 were subjected to PCR detection. Primer pair GTCCAGACCGCCGACCTC/CAGCAGCAGAGTGACAG was designed from whole-genome shotgun sequences of strain A22732 (data not shown) for amplifying the DNA fragments containing the whole coding region of blaOXA-114, because all the available primers gave negative amplification. All ampiclons were sequenced on ABI 37300 Sequencer with the same primers for PCR.

Detection of carbapenemase activity. Activity of class A/B/D carbapenemases was determined by CarbaNP test with modifications. Overnight bacterial cell culture in the Mueller-Hinton broth was diluted 1:100 into 3 ml of fresh Mueller-Hinton broth, and bacteria were allowed to grow at 37°C with shaking at 200 rpm to reach an optical density (OD₆₀₀) of 1.0 to 1.4. If required, ampicillin was used at 100 μg/ml. Bacterial cells were harvested from 2 ml of the above culture, and washed twice with 20 mM Tris-HCl (pH 7.8). Cell pellets were resuspended in 500 μl of 0.1 M Tris-HCl (pH 7.8), and lysed by sonication, followed by centrifugation at 10000 x g at 4°C for 5 min. 50 μl of the supernatant (the enzymatic bacterial suspension) were mixed with 50 μl of substrate I, V, respectively, followed by incubation at 37°C for a maximum of 2 h. Substrate I: 0.05% red phenol plus 0.1 mM ZnSO₄ (pH 7.8). Substrate II: 0.05% red phenol plus 0.1 mM ZnSO₄ (pH 7.8), 0.6 μg/ml imipenem. Substrate III: 0.05% red phenol plus 0.1 mM ZnSO₄ (pH 7.8), 0.6 μg/ml imipenem, and 0.8 mg/μl tazobactam. Substrate IV: 0.05% red phenol plus 0.1 mM ZnSO₄ (pH 7.8), 0.6 mg/ml imipenem, and 3 μM EDTA (pH 7.8). Substrate V: 0.05% red phenol plus 0.1 mM ZnSO₄ (pH 7.8), 0.6 mg/ml imipenem, 0.8 mg/μl tazobactam, and 3 μM EDTA (pH 7.8).

Conjugational transfer. Plasmid conjugational transfer experiments were carried out with rifampin-resistant E. coli EC600 being used as recipient and blaIMP-positive A. xylosoxidans as donors. 3 ml of overnight cultures of each of donor and recipient bacteria were mixed together, harvested and resuspended in 80 μl of Brain Heart Infusion (BHI) medium. The mixture was spotted on a 1 cm² filter membrane that was placed on the BHI agar plate, and then incubated for mating at 37°C for 12-18 h. Bacteria were washed from the filter membrane and spotted on the Muller-Hinton agar plate containing 1300 μg/ml rifampin and 100 μg/ml ampicillin for selection of the blaIMP-positive E. coli transconjugant.

Determination of minimum inhibitory concentration (MIC). The MIC values of indicated bacterial strains were tested by using VITEK 2 according to manufacturer’s instructions, and antimicrobial susceptibility was judged by Clinical and Laboratory Standards Institute (CLSI) standard.

Determination of plasmid DNA sequence. The chromosome DNA-free plasmid DNA was isolated from the cell cultures of the blaIMP-positive E. coli transconjugant using a Qiagen large construct kit, and then sequenced by using whole-genome shotgun strategy in combination with Illumina HiSeq 2500 sequencing technology. The contigs were assembled with Velvet, and the gaps were filled through combinatorial PCR and Sanger Sequencing on ABI 37300 Sequencer. The genes were predicted with GeneMarkS and further annotated by BLAST against Uniprot and NR databases.

RNA isolation and reverse transcription (RT)-PCR. Bacteria were cultivated overnight in Mueller-Hinton broth (BD) with or without addition of 2 μg/ml imipenem (Sigma). Total RNA was extracted from harvested bacterial cells using TRIzol Reagent (Life Technologies). RNA quality was monitored by agarose gel electrophoresis, and RNA quantity was determined by spectrophotometry. The contaminated DNA in the total RNA samples was removed by using Ambion’s DNA-free Reagent Kit. cDNAs were generated by using 5 μg of RNA and 3 μg of random hexamer primers in a 40 μl reaction mixture. The cDNA samples were generated by RT from total RNAs. Genomic DNA and cDNA were used as the templates for PCR and RT-PCR, respectively, with the primer pair TGGTTGATGTGCGAGGCAAGGCGTTAACAGTTGACAGCT to detect the particular IMP-positive E. coli strain. Reaction containing primer pairs without templates were also included as blank controls.

Primer extension assay. The [γ-32P] ATP end-labeled primer CAGCATAACGACGAT or CACATCTTTCCTTTTCTAAGCG, which was complementary to aacA7 or bleIMP-1 transcript, respectively, was annealed with total RNA sample of strain A22732 for primer extension assay as described previously31. For different cell cultures (lanes) in a single experiment, equal amounts of total RNA were used as starting materials. The corresponding end-labeled primer was also used for sequencing the PCR amplicon generated by the primer pair TGGTTGATGTGCGAGGCAAGGCGTTAACAGTTGACAGCT to detect the particular IMP-positive E. coli strain. Reaction containing primer pairs without templates were also included as blank controls.

Nucleotide sequence accession number. The complete sequence (File S1) of plasmid pA22732-IMP was submitted to the GenBank nucleotide sequence database under accession number KJ887880.
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Acknowledgments
This work is funded by National Key Program for Infectious Disease of China (2013ZX10004216, and 2013ZX10004217-002), National Basic Research Program of China (2014CB744000), Program of Manned Spaceflight (040203), and National Natural Science Foundation of China (81350020, and 81373077). All the experiments were done in Dr. Dongsheng Zhou’s laboratory.

Author contributions
D.Z. and C.L. designed experiments. Z.C., H.F., L.W., P.S., Y.W., Z.Y., H.Y., W.Y., J.P., P.X., D.Z. and C.L. performed experiments. D.Z., Z.C. and H.F. analyzed data. D.Z., Z.C., H.F. and Y.W. contributed reagents, materials and analysis tools. D.Z. and C.L. wrote this manuscript.

Additional information
Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Chen, Z. *et al.* IMP-1 encoded by a novel Tn402-like class 1 integron in clinical *Achromobacter xylosoxidans*, China. *Sci. Rep.* 4, 7212; DOI:10.1038/srep07212 (2014).