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経腸栄養法に関する感染対策

井上　善文

1. 経腸栄養チューブ留置に関連した感染対策

1.1 経鼻チューブ留置時の副鼻腔炎や中耳炎の予防

1.1.1 5～12Fの口径の経腸栄養専用チューブを用いる方が良い。[1-3]（IIIB）

1.1.2 長期留置の場合は胃瘻や腸瘻へ変更する方が良い。（IIIC）

1.2 経鼻チューブ挿入時の気管内誤挿入の予防

1.2.1 経腸栄養用経鼻チューブ挿入後には先端位置をX線撮影で確認する。[4-8]（IIIA）

1.3 胃瘻造設に関連した瘻孔部感染予防対策

1.3.1 咽頭部の細菌を胃瘻造設部に定着させない工夫、ストッパーによる胃壁に対する過度の圧迫を避ける工夫をする方がよい。[9]（IIIB）

1.3.2 胃瘻周囲の状態を毎日観察し、清潔な状態を保つ方が良い。[10]（IIIB）

2. 細菌性腸炎予防対策

2.1 経腸栄養剤調製時の注意点

2.1.1 感染予防のためには、バッグ型：RTH（Ready-To-Hang）製剤を用いる方が良い。[11-15]（IIB）

2.1.2 調製する必要がある経腸栄養剤は、投与直前に調製する方が良い。[16]（IIB）

2.1.3 経腸栄養剤を調製後、投与までに時間がある場合は冷蔵庫内に保存する方が良い。（IIIB）

2.1.4 開封した後、冷蔵していない状態で8時間以上経過したものは廃棄する方が良い。[17]（IIIB）

2.2 経腸栄養剤投与時の注意点

2.2.1 溶解・希釈を行う製剤では8時間以内に、RTH製剤では24時間以内に投与を完了する。[18-21]（IIA）

2.2.2 経腸栄養剤投与容器は使用のたびに洗浄・消毒し、経腸栄養剤の注ぎ足しをしない方が良い。[21, 22]（IIIB）

2.2.3 経腸栄養投与ラインは、使用するたびに洗浄・消毒を行う方が良い。[24-28]（IIIB）

2.2.4 空腸瘻から経腸栄養剤を投与する場合は、胃瘻を介した場合よりも厳重な清潔操作を行う方が良い。[29, 30]（IIIB）

3. 誤嚥性肺炎防止対策

3.1 胃瘻からの経腸栄養で誤嚥性肺炎が発生する場合は、腸瘻から投与する方が良い。[31-46]（IIIB）

3.2 経腸栄養剤投与時には、誤嚥性肺炎防止のために上半身を挙上する方が良い。[47-50]（IIIB）

3.3 急速に胃内に経腸栄養剤を注入することにより胃食道逆流に伴う誤嚥性肺炎を起こす危険があるので、徐々に投与速度をあげる方が良い。[51-53]（IIIB）

3.4 経腸栄養剤の固形化・半固形化も考慮しても良い。[54, 55] (IIIC)

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内視鏡感染防止対策

1 内視鏡室

1.1 施設で内視鏡検査を施行する部門を一カ所に集約する方が良い（内視鏡センター）[1]（IIIB）

1.1.1 内視鏡の衛生管理と保守点検の責任者をおく。（IVA）

1.1.2 内視鏡の運用（洗浄・消毒、個人防護具の使い方、内視鏡の保管、検査室の清掃など）に関する手順を標準化する。（IIIA）

1.2 施設に適合した内視鏡の運用方法をマニュアル化する（IVA）

1.2.1 使用するたびに製造業者の取扱説明書に従って、圧力／リーク・テストを実行する。[1-4]（IA）

1.2.2 使用後の内視鏡はコンテナに入れ周囲に汚染のないように洗浄室まで搬送する。（IIIA）

1.3 内視鏡室は、医療従事者や患者の安全を考慮して設計され、換気設備により有害な消毒剤の曝露を最小限化する。[1, 4-9]（IA）

1.4 術者、介助者、洗浄する者が、個人防護具をいつでも使用できるようにして、化学物質、血液、他の感染性物質に曝露されないようにする。[10-12]（IIIA）

1.4.1 術者は、検査中に清潔な手袋（未滅菌でよい）、ガウン、マスク、ゴーグル（またはフェースシールド）を着用する。

1.4.2 検査終了後、個人防護具をすべて外し、手指消毒をおこなう。

1.4.3 個人防護具をしたままカルテなどの記載はおこなわない。

1.4.4 介助者は、必要に応じて個人防護具を使用するが、患者毎に個人防護具を変える。

1.4.5 洗浄する者は、手袋、ガウン、マスク、ゴーグル（またはフェースシールド）を着用する。

1.4.6 洗浄終了後、個人防護具をすべて外し、手指消毒をおこなう。

1.4.7 洗浄中であっても個人防護具をしたまま検査室から出ない。

1.5 内視鏡室に勤務するすべての職員は、感染管理上の推奨事項（例えば標準予防策）について訓練を受け、それを厳守する。[13]（IIIA）

1.5.1 内視鏡の衛生管理に関する院内研修会を開催する。（IVA）

1.5.2 独自に院内研修会を持ちにくい施設では、地域で連携して内視鏡の衛生管理に関する院外研修会に参加する。（少なくとも年1回以上）（IVA）

1.6 内視鏡が使用前のものか使用後のものか判別できるよう、医療機関で取り決めをしておく。（IIIA）

1.6.1 内視鏡の保管、検査室、洗浄室の順で動線を設定し、使用前の内視鏡と使用後の内視鏡が交差しないようにする。

1.6.2 使用後の内視鏡は直ちに専用の搬送用トレイに入れ、洗浄室に搬送する。

1.7 患者名、診療録番号、手技名、術者、内視鏡シリアルナンバー、洗浄者、内視鏡洗浄消毒装置についての検査記録簿を作成し記録する。[1, 5]（IVA）

2 内視鏡の一次洗浄

2.1 内視鏡の部品（送気・送水と吸引バルブなど）を取扱説明書に従って取り外し、完全に酵素系洗剤に浸漬する。[14, 15]（IIIA）

2.2 酵素系洗剤は、使用毎に廃棄する。[2, 4, 15]（IIIA）

2.3 自動洗浄消毒器を使用する場合でも必ず一次洗浄を行う。[1-5, 16-26]（IIIA）

2.4 内視鏡のチャンネル、部品、コネクタは開口部の大きさに合ったブラシを用いて洗浄する。[4, 21, 26]（IIIA）

2.4.1 洗浄用品は、ディスポーザブル製品にするか、使用毎に洗浄後、消毒する。[4, 21, 26]（IIIA）

3 内視鏡の再処理（消毒／滅菌）

3.1 内視鏡は、使用毎に高水準消毒を行うか滅菌する。[1, 2, 4, 5, 13, 16, 19, 21, 26, 27]（IIIA）

3.1.1 高水準消毒薬として、グルタールアルデヒド、フタラール、過酢酸を使用する。（IIIA）

3.1.2 内視鏡に適合した消毒薬を取扱説明書に従って選択する。[2, 4, 5, 21, 28-32]（IIIA）

3.2 再利用可能な生検鉗子は滅菌する。[1-5, 13, 16, 19, 21, 33, 34]（IIIA）

3.3 浸漬法を行う場合、内視鏡や部品を高水準消毒薬に完全に浸漬する。高水準消毒薬が全てのチャンネルを満たしていることを確認する。[1, 3-5, 19, 21, 28-30]（IB）

3.4 取扱説明書により内視鏡と自動洗浄消毒器の適合性について確認する。[2, 5, 21, 28-32]（IB）

3.5 用手法で消毒する場合、高水準消毒の後に、滅菌水、濾過水のいずれかで内視鏡のすすぎチャンネルを洗い流して、消毒薬を除去する。[1-3, 5, 19, 23, 35-38]（IIIA）

3.5.1 内視鏡をすすいだ水は一回毎に排水する。[1-3, 5, 19, 23, 35-38]

3.5.2 チャンネルに70～90%のエタノールまたはイソプロパノールを通した後、送気して乾燥させる。[1-3, 5, 19, 23, 35-38]

3.6 高水準消毒薬については最小有効濃度を日常的に確認する。[1, 2, 4, 5, 19, 27, 39]（IIIA）

3.6.1 使用開始前に消毒薬をチェックして結果を記録する。

3.6.2 化学的インジケータで有効濃度を下回っていた場合、その消毒薬は廃棄する。[1, 2, 4, 5, 19, 27, 39]

3.6.3 自動洗浄消毒器に消毒薬をつぎ足す場合は、最初に入れた溶液を基準として期限を設定する（用手洗浄の場合も同様）。消毒薬を後からつぎ足しても、使用期限は延長しない。[4, 19, 40]

3.7 送水ボトルとその連結チューブは、最低一日一回高水準消毒または滅菌を行う。送水ボトルには滅菌水を入れる。[1, 41-44]

4 内視鏡の保管

4.1 内視鏡は、汚染しないように保管する。[1, 4, 19, 21]（IIA）

4.1.1 洗浄、消毒した内視鏡は、専用の保管庫で保管する。（ケースに保管しない）

4.1.2 保管庫のドアは閉めておく。

4.2 内視鏡は、乾燥しやすいように垂直に立てて保管する。（製造元の指示に従ってキャップ、弁、他の取り外し可能な器具は外しておく。）[1, 2, 4, 19, 21, 45]（IIA）

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病原体別感染拡大防止策

荒川　宜親

1 多剤耐性菌

原則：多剤耐性菌による感染症を防止、低減化させる為には、①耐性菌の早期検出、②感染源や感染経路の特定と予防策による伝播・感染拡大の防止、③抗菌薬の使用法に関する点検と見直し、の三点が重要であるが、②と③については、別章で詳しく論じられるため、本章では、骨子のみを記述する。

1.1 バンコマイシン耐性腸球菌：VRE

1.1.1 VREによる感染症患者を減少または患者予後を改善するためは、保菌率を下げる方が良い[1,5]。(IIB)

1.1.2 ハイリスク患者を収容、治療する骨髄移植病棟などではVRE保菌者のスクリーニングと汚染・感染防止策（標準的な感染予防策、接触感染予防策）を実施する方が良い[6-9]。(IIB)

1.1.3 長期抗菌薬使用患者では定期的便培養を行う方が良い[10]。(IIB)

1.1.4 VREの保菌者の多いICUでは、汚染・感染防止策をとる[11-13]。(IIA)

1.1.5 第三世代セファロスポリンやバンコマイシンの投与は、術後のVRE感染症のリスク因子になるため、予防投与は避けた方が良い[14-16]。(IIB)

1.2 メチシリン耐性黄色ブドウ球菌：MRSA

1.2.1 MRSA感染症を低下させるには、MRSAの保菌のスクリーニングをした方が良い[17]。(IIB)

1.2.2 MRSA感染症を低下させるには、保菌者、感染症患者の個室収容、汚染・感染防止策（標準的な感染予防策、接触感染予防策）を行う[18,19]。(IIA)

1.3 多剤耐性緑膿菌：MDRP

1.3.1 多剤耐性緑膿菌の感染症は、ICU患者の予後を悪化させるので、その発生や拡散を予防する[20-24]。(IIA)

1.3.2 抗菌薬の長期投与は多剤耐性緑膿菌の選択や定着を促進するため、行わない[25]。(IIA)

1.4 薬剤耐性菌全般

1.4.1 薬剤耐性菌による感染症を減らす為には、看護師の配置を十分に行う方が良い[26,27]。(IIB)

2 結核菌

2.1 救急外来、剖検室で、結核が疑われる患者に接する職員は、N95マスクを着用する。(IIIA)

2.2 外来の予診で結核が疑われる患者については、トリアージ（患者の予診に基づく疑似患者の選り分けと個室待機、優先診療）を行う。(IIIA)

2.3 活動性の結核症およびそれが疑われる患者を確認した場合は、医師は、直ちに、最寄りの保健所長に報告し、「結核症指定医療機関」に紹介し、転送する。(IVA)

2.4 活動性の結核患者に接した職員には、健康診断と予防投薬を行う。(IVA)

2.5 転送が困難な重症の排菌患者は、陰圧空調の個室に収容し、患者に接する際は、手指衛生の励行とN95マスクを着用する。(IVA)

2.6 多剤耐性結核菌（MDR-TB、XDR-TB）と判定された場合は、直ちに、「結核症指定医療機関」の専門家に相談し対策を講じる。(IVA)

2.7 結核菌またはそれを含む臨床材料を扱う場合は、必要な防護具を付け安全キャビネットの中で操作する。(IVA)

3 バチルス属菌等非侵襲性環境細菌

3.1 バチルス属菌等の環境菌が血液培養で分離された場合は、輸液（ルート）、静脈注射薬などの汚染による可能性を第一に考慮し、調査と対策を行う[28,29]。(IIA)

3.2 バチルス属菌等の環境菌の、同時多発事例や急激な分離件数の増加を確認した場合は、環境汚染調査を実施する方が良い。(IIIB)

4 飛沫感染、飛沫核感染（＝空気感染）で伝播するウイルス等

4.1 麻疹、水痘、インフルエンザ、SARS等、飛沫感染や飛沫核感染（＝空気感染）で伝播拡散する病原体による院内感染には、伝播様式に応じた感染予防策を実施する。(IVA)

4.2 外来では、疑似患者については、トリアージを行う。(IIIA)

4.3 空気感染の感染源となりうる患者を入院させる場合は、陰圧空調の個室に収容する。(IVA)

4.4 SARSについては、疑似患者の段階で、「患者」とみなして必要な法的手続きと感染拡大防止策を講じる。(IVA)

5 ヒト-ヒト感染が極めて低いとされている病原体

5.1 レジオネラ、アスペルギルス、非結核性抗酸菌など、ヒトからヒトへ感染する頻度が極めて低いとされている病原体による感染症については、一般的な感染予防策は無用である。(IIIA)

5.2 給水（湯）設備やシャワーの蛇口などでレジオネラが繁殖しないよう、定期的な消毒とともに、水温を20℃以下、遊離残留塩素濃度を0.2～0.4mg/L程度に常時保つか、貯湯槽で60℃以上、吹き出し口で55℃以上の熱湯を給湯する[30,31]。(IVA)

5.3 レジオネラ感染症が施設内で発生した場合は、浴槽、リハビリ用プール、シャワーの蛇口などの湿潤箇所の培養検査と検出箇所の使用禁止、衛生管理を徹底する。(IIIA)

5.4 レジオネラの院内感染防止には、銅-銀イオン化システムを利用する方が良い[32]。(IIB)

6 *Clostridium difficile*、ノロウイルスなど糞便、吐物を介して感染する病原体

6.1 *C. difficile*：患者は個室収容かコホーティングを行い、汚染・伝播防止策（標準的な感染予防策、接触感染予防策）の徹底、排便介助、オムツ交換、糞便処理の際の手指衛生の徹底、通常洗剤により、手が触れる箇所の定期的拭き取りによる芽胞の物理的除去、高濃度汚染が疑われる場合は、次亜塩素酸Naなどを用いて消毒する。(IIA)

6.2 ガチフロキサシン、モキシフロキサシンの投与後に分離された株は、北米で流行している強毒型のBI/NAP1/027株か否か検査する方が良い。(IIB)

6.3 重篤な腸管感染症状を呈する患者では強毒型のBI/NAP1/027株を想定し、培養検査を行う方

が良い。(IIB)

6.4 ノロウイルスでは患者は個室収容かコホーティングし、汚染・感染防止策（標準的な感染予防策、接触感染予防策）を徹底する。(IIIA)

6.5 下痢、嘔吐物の処理時には、次亜塩素酸 Na(塩素濃度 200ppm)などの消毒剤を使用する[33]。(IIA)

7 消毒薬に抵抗性を示す細菌

7.1 *C. difficile*、バチルス属菌など芽胞を形成する菌種に対しては、一般の消毒処置が無効であるため、手が触れやすく、汚染されやすい箇所を通常洗剤を用いて定期的に物理的な拭き取りによる除染を行う。(IIA)

7.2 芽胞の汚染が想定される場合には、次亜塩素酸 Na を含む消毒剤を用いた消毒を行う。(IIA)

7.3 クロルヘキシジンに抵抗性を示す *Burkholderia cepacia* やブドウ糖非発酵菌群によるアウトブレークが発生した場合には、消毒薬の使用が使用説明書通りに行われているかの点検を行い、湿潤箇所の拭き取り検査、さらに消毒薬抵抗性株の出現を考慮して対策を行う方が良い。(IIIB)

8 食品を介して感染する可能性のある病原体

8.1 ノロウイルス、サルモネラ、腸管出血性大腸菌（O157 など）カンピロバクターなど、汚染された食品を介して感染する可能性のある病原体による感染症が同時多発した場合は、食中毒とともに院内感染の両面からの調査と対策を実施する。(IVA)

8.2 ノロウイルス：前述を参照。

8.3 サルモネラ、腸管出血性大腸菌（O157 など）など：標準的な感染予防策の励行、可能な場合は個室収容、下痢便、吐物の処理の際の汚染・感染拡大防止策を徹底する。(IVA)

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アウトブレイク対応策

鈴木　里和

1 対応組織

1.1 院内感染対策委員会

1.1.1 院内感染対策委員会はアウトブレイクが疑われる場合に報告を受ける体制を整備しておく。(IVA)

1.1.2 施設管理者または院内感染対策担当責任者はアウトブレイクが疑われる場合、随時に院内感染対策委員会を開催する。(IVA)

1.2 外部調査委員会

1.2.1 院内感染対策委員会では感染源・感染経路の特定が困難な場合は保健所などの行政機関、関連学会などの協力のもと外部調査委員会を設置する。(IIIA)

1.2.2 外部調査委員会は院内感染対策委員会の感染源・感染経路に関する調査結果と対策の妥当性を評価し、改善策を提言した後に、公開する。(IIIA)

2 対応の基本手順

2.1 アウトブレイクの発生を疑った場合、以下の手順で対応を進める。[1] (IIIA)

① アウトブレイクの確認
② 範囲（病棟・期間）の確認とアウトブレイク症例の確定
③ 対応策の提示と実施
④ 感染源・感染経路に関する調査
⑤ アウトブレイク収束の確認

3 アウトブレイクの確認

3.1 アウトブレイクを疑う情報を収集できる報告システムを構築する。(IVA)

3.2 院内感染対策委員会はアウトブレイクを疑った場合、過去の発生状況及び原因病原体の分離や患者の迅速診断、血清診断結果に基づきアウトブレイクの発生を確認する。(IIIA)

4 アウトブレイクの範囲とアウトブレイク症例の確定

4.1 院内感染対策委員会が未把握の感染症 / 無症状病原体保有患者の有無を以下の方法で確認し、対応および調査の対象とする病棟及び期間を定める。(IIIA)

4.1.1 薬剤耐性菌による事例の場合には[1]保菌検査を行う。(IIIA)

4.1.2 薬剤耐性菌の保菌検査は症例と同一の看護単位の全入院患者を対象とした方が良い。(IIIB)

4.1.3 アウトブレイク症例を明確にするため、アウトブレイク症例の定義を作成する (IIIA)

4.1.4 アウトブレイク症例の定義には①アウトブレイクの対象となる感染症・無症状病原体保有患者の定義、②アウトブレイクの発生期間、③アウトブレイクの対象となる病棟、の3項目を含める方が良い。(IIIB)

5 対応

5.1 初期対応

5.1.1 アウトブレイクの原因病原体の伝播経路に応じて症例の隔離を実施する。(IIIA)

5.1.1.1 処理病原体に応じた設備が整備された病室で個室またはコホート管理を実施する。(IIIA)

5.1.2 対象となる病棟のすべての入院患者の湿性体液物質に触れる処置をする際には手袋・マスク・ガウン等の使用と手指の衛生管理を徹底・強化する。(IIIA)

5.1.3 対象となる病棟における手指衛生管理の実施を徹底・強化する。(IIIA)

5.1.4 症例の隔離のため職員に対する感染対策に関する情報提供を行った方が良い。(IIIB)

5.1.5 医療器材の滅菌と消毒・使用薬剤の衛生管理および処置時の衛生管理を再度見直す。(IIIA)

5.1.6 予防薬やワクチンがある場合は、その適応を考慮した方が良い。[2,3] (IIIB)

5.2 初期対応後の対応

5.2.1 初期対応の有効性を評価するため、新規のアウトブレイク症例の有無を継続的に監視する。(IIIA)

5.2.2 新規のアウトブレイク症例が発生した場合、初期対応を講じた後も病原体の伝播が阻止されていない可能性があるので以下の対応をする。

5.2.2.1 感染源、感染経路に関する調査を実施し随時対策を追加する。(IIIA)

5.2.2.2 感染源、感染経路の特定が困難な場合は以下のような外部専門家の支援を要請する。(IIIA)

① 保健所や地方衛生研究所
② 各都道府県を通じて要請する国立感染症研究所実地疫学専門家養成プログラム（FETP）
③ 院内感染地域支援ネットワーク事業
④ 大学等の医育機関
⑤ 感染症関連学会等

5.2.2.3 当該病棟への新規入院の中止等、診療を制限した方が良い。[4] (IIIB)

5.3 事例後対応

5.3.1 継続的な監視を行っても新規の症例発症が一定期間*認められなかった場合にアウトブレイクの収束と判断して良い。(一定期間：一般的には潜伏期間の2～3倍の期間) (IIIC)

5.3.2 アウトブレイクの収束が確認された後、感染源、感染経路に関しての調査結果を参考に、一時的に強化していた種々の対策を継続可能な対策に切り替えていく。(IIIA)

6 調査

6.1 事例の早期収束および再発防止のため、アウトブレイク症例に共通する感染源・感染経路に関する調査を行う。(IIIA)

6.2 病原体・患者検体の確保および検査

6.2.1 分離された病原体や採取された血清などの検体は保管する[5,6]。(IIIA)

6.2.2 環境消毒の前に環境調査を行なう。(IIIA)

7 情報の公開

7.1 事例の概要、感染源・感染経路に関する調査内容、改善策をまとめ、患者の了解を得た後に公開する。(IIIA)

8 患者・家族・医療従事者への情報提供

8.1 入院患者およびその家族に対しては、初期対応時に診療担当者と院内感染対策の担当者等から十分な情報を提供し対策に必要な協力を要請する。(IIIA)

8.2 すべての職員に対して、院内感染対策委員会よりアウトブレイク事例ならびにその対策に関する情報を提供する。(IIIA)

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Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

著者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Wachino J, Yamane K, Kimura K, Shibata N, Suzuki S, Ike Y, Arakawa Y	Mode of transposition and expression of 16S rRNA methyltransferase gene *rmtC* accompanied by IS*Ecp1*	Antimicrob Agents Chemother.	50(9)	3212-3215	2006
Park YJ, Lee S, Yu JK, Woo GJ, Lee K, Arakawa Y	Co-production of 16S rRNA methylases and extended-spectrum β-lactamases in AmpC-producing *Enterobacter cloacae*, *Citrobacter freundii* and *Serratia marcescens* in Korea.	J. Antimicrob. Chemother	58(4)	907-8	2006
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Mode of Transposition and Expression of 16S rRNA Methyltransferase Gene *rmtC* Accompanied by IS*Ecp1*

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A newly identified 16S rRNA methyltransferase gene, *rmtC*, was accompanied by an IS*Ecp1* element at its 5′ end. This IS*Ecp1* element, which contained a transposase gene, *tnpA*, provided a promoter activity for expression of the adjacent *rmtC*; and this structure enabled the *rmtC* gene to be transposed onto another plasmid in *Escherichia coli*.

Four types of plasmid-mediated 16S rRNA methyltransferase genes, *rmtA*, *rmtB*, *rmtC*, and *armA*, which confer high levels of resistance to various aminoglycosides, have been found worldwide among a number of pathogenic gram-negative rods (3, 4, 8, 14, 17–19). The distribution of these plasmid-mediated 16S rRNA methyltransferase genes among pathogenic bacteria seems attributable to the fact that these genes are associated with some bacterium-specific DNA recombination systems, such as a transposon (3, 5, 7, 16). In fact, it was recently reported that transposition of *armA* was mediated by a composite transposon, Tn*1548* (5). However, little is known about the transposition system of the other three plasmid-mediated 16S rRNA methyltransferase genes, *rmtA*, *rmtB*, and *rmtC*. Therefore, in the present study we characterized in detail the transposition system of *rmtC*, which was located on a plasmid (pARS68) found in a clinical *Proteus mirabilis* strain, ARS68 (14).

A SacI-digested 11-kb fragment carrying *rmtC* was cloned from pARS68, and both strands were entirely sequenced. By using the bacterial genetic code, ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to search for open reading frames (ORFs). A schematic representation of the cloned fragment is shown in Fig. 1A. IS*Ecp1*, which contained a transposase gene, *tnpA*, was located just upstream of *rmtC*. Although there were several ORFs (*orf1* to *orf8*) around IS*Ecp1* and *rmtC*, their functions remained unknown, even though their sequences were compared with the sequences in the public databases of GenBank and EMBL by using the BLAST and FASTA search tools.

It is well known that IS*Ecp1* is often located at the 5′ ends of several β-lactamase genes, such as $bla_{CTX\text{-}M}$ and bla_{CMY} (1, 2, 6, 9, 11, 12, 15), and enables these genes to be transposed to other DNA target sites (2, 10, 13). Moreover, IS*Ecp1* provides promoter activity for expression of a downstream CTX-M-type β-lactamase gene (2, 12). These findings strongly suggested that the transposition and expression of *rmtC*, as seen in pARS68, were also regulated by IS*Ecp1*. IS*Ecp1* was bracketed with two imperfect 14-bp inverted repeat (IR) sequences (the left IR [IRL] and the right IR [IRR]) (Fig. 1A). A putative 5-bp target site (TTCAA) was located in the immediate vicinity of IRL (Fig. 1A). This 5-bp target site might be duplicated, most likely after the insertion of a DNA fragment mediated by a transposon, and is subsequently located on both faces of a transposed DNA fragment. The presence of duplicated 5-bp target sites can be a trace that the insertion of a DNA fragment by transposon occurred at that position. Considering the flanking genetic organizations of *rmtC*, it is speculated that a DNA fragment containing IS*Ecp1* and *rmtC* bracketed on one side by IRL and on the other by a putative second IRR, IRR′, constituting a potential transposon on pARS68. To determine if *rmtC* could transpose with IS*Ecp1* and to determine the structural limits of the transposable unit, we tried to identify a potential transposon carrying IS*Ecp1* and *rmtC* bracketed with IRL and putative IRR′ by an in vitro transposition experiment. For this purpose, the transposition of *rmtC* carried by several donor recombinant plasmids based on two different backbones (Fig. 1A) to the recipient plasmid R388 (TMP^r) was investigated with a standard mating assay. *Escherichia coli* DH5α harboring R388 together with various recombinant plasmids and *E. coli* HB101 (STR^r) were used as the donor and recipient strains, respectively. Transconjugants were selected on Luria-Bertani agar plates supplemented with gentamicin (10 μg/ml), trimethoprim (50 μg/ml), and streptomycin (100 μg/ml). Transconjugants were obtained when *E. coli* DH5α carrying recombinant plasmids (pBCS68, pBCHS68, pMCLS68, and pMCLHS68) (Fig. 1A) was used as a donor strain at a frequency of ca. 10^{-7} to 10^{-6} per recipient. On the other hand, transconjugants could not be obtained (frequency, $< 10^{-9}$ per recipient) when *E. coli* DH5α carrying plasmids (pBCES68 and pMCLES68) lacking a part of IS*Ecp1* was used (FIG. 1A). These findings strongly suggested that IS*Ecp1* plays an essential role in the transposition of *rmtC*.

Twenty recombinant plasmids carrying *rmtC* with the backbone of plasmid R388 obtained when pBCS68 was used as a donor plasmid were extracted from the transconjugants. The sequences of both terminal ends of the transposed fragments

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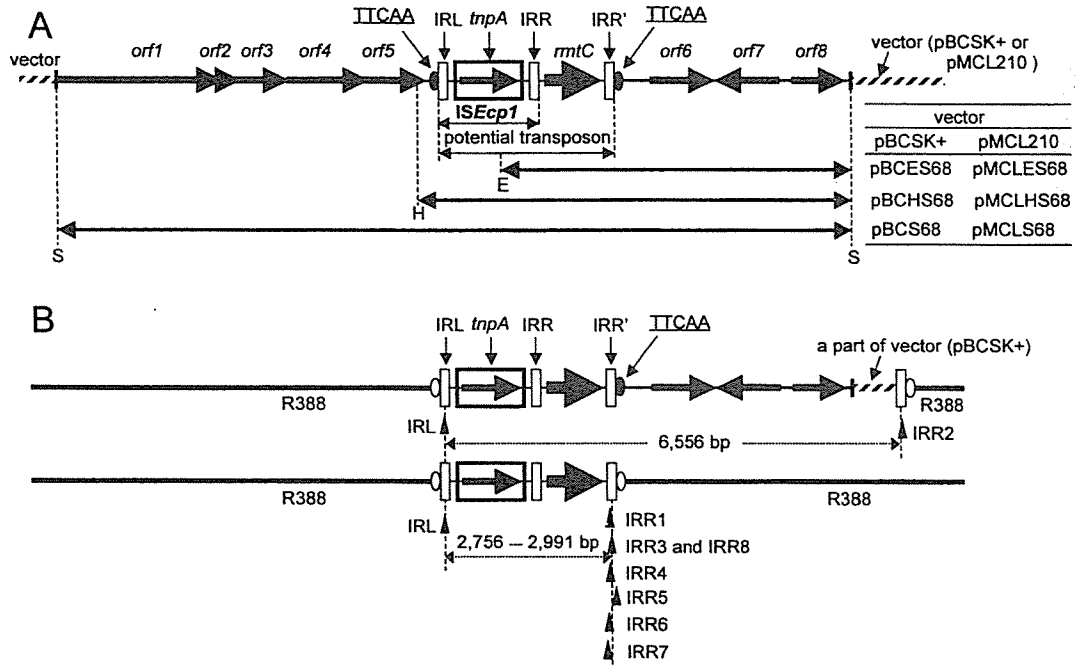

FIG. 1. (A) Schematic organization of genes in the 11-kb SacI fragment of pBCS68. ORFs and genes are shown as arrows indicating transcription orientation. The IRL, IRR, and IRR′ motifs are indicated by open rectangles. Possible 5-bp target sites (TTCAA) are shown as a closed ellipse. DNA fragments carried by recombinant plasmids used in the transposition experiments are indicated by double-headed arrows with the corresponding recombinant plasmid names. Fragments on pBCHS68 and pMCLHS68 were generated by ligating the fragment on pBCES68 or pMCLES68, and the PCR fragments were amplified by using primers supplemented with restriction sites (HindIII or EcoRI). Restriction sites: S, SacI; H, HindIII; E, EcoRI. (B) Transposition of the IS*Ecp1-rmtC* element onto R388 from pBCS68. The positions of the IRRs (IRR1 to IRR8) were confirmed by transposition experiments with the IS*Ecp1-rmtC* element in *E. coli* cells by using R388 as the recipient plasmid. Positions of the 5-bp duplicated target sites are shown as open ellipses. Upward-pointing arrowheads indicate the positions of the IRL and the IRR′ extremities of each probable transposed fragment (horizontal broken lines). This result reveals that the IRR′ elements of IS*Ecp1* are not so rigorous but are comparatively multifarious or flexible and that the 5-bp target sites or recombination junctions at the IRR′ side are also diverse.

were determined in detail by direct sequencing of these recombinant plasmids with customized primers. As a result, eight types of transposed fragments, which were structurally different from each other, were obtained. All transposed fragments analyzed contained both IS*Ecp1* and *rmtC*, and each left end (IRL) of those fragments was perfectly identical (Fig. 1B). However, the right end of each fragment (IRR1 to IRR8) varied (Fig. 1B and Table 1). IRR was within 459 bases of the end of *rmtC* in seven of eight cases, although only IRR2 belonged to the cloning vector region (Fig. 1B). The locations of IRR3 and IRR8 were adjacent to the typical 5-bp nucleotide sequence, TTCAA, which seemed to be an innate target site on pARS68. Therefore, it is probable that the 2,973-bp fragment bracketed on the left side by IRL and on the right end by IRR3 or IRR8 constituted a potential transposon on plasmid pARS68.

TABLE 1. Characteristics of inverted repeats and target sites for IS*Ecp1*-mediated transposition

Description of sequence	Nucleotide sequence of IRL and IRRs (14 bp)[a]	5-bp duplicated target site sequence	Size of transposed fragment (bp)
IRL of IS*Ecp1*	5′-CCTAGATTCTACGT-3′	TTCAA	
Expected perfect IRR of IS*Ecp1* (complementary sequence of IRL)	5′-ACGTAGAATCTAGG-3′		
IRR of IS*Ecp1*	5′-ACGTGGAATTTAGG-3′		
IRR′	5′-CCTAGGAACTCGGC-3′	TTCAA	2,973
IRR1	5′-GCCTGGGATTTCGA-3′	TTCTT	2,943
IRR2	5′-GAACAGTATTTGGT-3′	TATGT	6,556
IRR3	5′-CCTAGGAACTCGGC-3′	ACGCA	2,973
IRR4	5′-TCCTAGGAACTCGG-3′	GCCAA	2,972
IRR5	5′-ATATGGTGTTTCCT-3′	ATGAA	2,991
IRR6	5′-AATCTTTTTTCGGA-3′	AATTT	2,774
IRR7	5′-ACGCCGTAACTCGG-3′	GTGAC	2,756
IRR8	5′-CCTAGGAACTCGGC-3′	GAAAT	2,973

[a] Underlining of IRRs indicates nucleotide residues identical to the corresponding complementary nucleotide residues of the IRL sequence. IRR′, IRR3, and IRR8 showed identical nucleotide sequences.

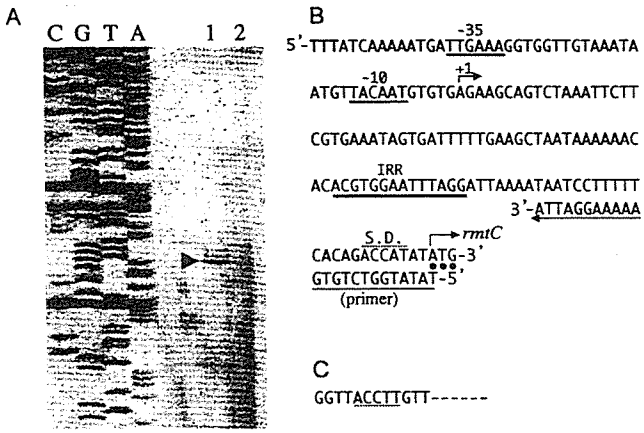

FIG. 2. Identification of transcriptional start site for *rmtC* in *E. coli* DH5α(pBC-KB1) by primer extension analysis (14). (A) Lanes C, G, T, and A, results of sequencing reactions performed with DNA prepared by PCR as a template and the primer; lane 1, primer elongation product obtained with total RNA of *E. coli* DH5α(pBC-KB1); lane 2, primer elongation product obtained with total RNA of *E. coli* DH5α (pBCSK+). (B) Promoter region of *rmtC*. +1, transcriptional start site for *rmtC*; putative −35 and −10 promoter sequences upstream of transcriptional start site are underlined with a thin line; dots indicate the ATG start codon of *rmtC*; a probable Shine-Dalgarno (S.D.) sequence is marked with a dotted line; the IRR of IS*Ecp1* is underlined with double thin lines; the primer used in primer extension analysis shown with an arrow at bottom. (C) Complementary sequence of the 3′ terminus of the 16S rRNA of *Proteus mirabilis*. The portion of the nucleotide residues that coincide with the upstream region of ATG start codon of *rmtC* is marked with a dashed line. The sequence was referred to the EMBL/GenBank database and can be found under accession no. AJ301682.

The numbers of base pairs in IRR, which is identical to those in IRL, ranged from three to nine (Table 1). The 3′ ends of the IRRs identified varied (GA, GT, GC, GG, or CT), although it was reported that IS*Ecp1B* needs a guanosine (G) residue at the 3′ ends of the IRRs when it transposed the adjacent genes bla_{KLU-A} and $bla_{CTX-M-19}$ (10, 13). In any event, it was commonly observed that IS*Ecp1* and IS*Ecp1B* were able to transpose adjacent antibiotic resistance genes by using IRRs composed of a wide variety of nucleotide sequences.

Primer extension analysis of RNA from RmtC-producing *E. coli* transformant, *E. coli* DH5α(pBC-KB1) (14), revealed the start residue of the mRNA transcription of *rmtC* (Fig. 2A and 2B). Transcription was initiated at an A (adenine) residue, located 99 nucleotides upstream of an AUG translation initiation codon of *rmtC*. This position was located within IS*Ecp1* near its IRR. Although diversity in the start residue of transcription was observed among IS*Ecp1*-bearing antimicrobial resistance genes, including *rmtC*, bla_{CTX-M}, and bla_{CMY}, IS*Ecp1* commonly provides promoter sequences within the right-end region near its IRR for expression of downstream antibiotic resistance genes (2, 6, 12).

Recently, it was experimentally confirmed that IS*Ecp1B* could transpose upstream of chromosomally located bla_{KLU-A} of *Kluyvera ascorbata*, which is thought to be a progenitor of CTX-M type β-lactamases, and, consequently, could also transpose bla_{KLU-A} to other target sites in *E. coli* (10). This hybrid structure of IS*Ecp1B* and bla_{KLU-A} seems to be the origin of that of IS*Ecp1* and bla_{CTX-M}, which is widely distributed among members of the family *Enterobacteriaceae* worldwide. Although the overall schemes for the development of the hybrid structure of IS*Ecp1* and *rmtC* have not been elucidated, it is probable that IS*Ecp1* first transposed into the 5′ end of chromosome-carrying *rmtC* in unknown aminoglycoside-producing bacteria and that subsequently the IS*Ecp1-rmtC* element transposed to other DNA target sites on a residential plasmid of the *Enterobacteriaceae*. To understand the development of the hybrid structure of IS*Ecp1* and *rmtC*, it would be necessary to identify natural reservoirs of *rmtC*. In conclusion, we report here that IS*Ecp1* plays an essential role in the transposition and expression of a 16S rRNA methyltransferase gene, *rmtC*.

Nucleotide sequence accession number. The nucleotide sequence of the 11-kbp SacI fragment shown in Fig. 1A was submitted to the EMBL/GenBank database through the DNA Data Bank of Japan (DDBJ) and can be found under accession no. AB194779.

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isoleucine alteration in the naturally occurring enzyme.[6] A single amino acid substitution not corresponding to any naturally occurring β-lactamase was identified in the mutants derived from OXA-10 (Table 1). Danel *et al.*[4] also generated (with non-hypermutable *P. aeruginosa*) an OXA-derived ESBL from R151 that does not correspond to a naturally occurring enzyme.

Although ceftazidime resistance has been associated with hypermutability in clinical isolates of *P. aeruginosa* from chronic lung infections[2] it is not known whether these strains contain ESBLs. Indeed on the basis of data reported here, we suggest that hypermutable *P. aeruginosa* may not be the source of those ESBLs found in clinical isolates of this organism. This situation may reflect the relative ease by which derepression of AmpC in *P. aeruginosa* provides a route for resistance to expanded-spectrum β-lactams.

Transparency declarations

None to declare.

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Co-production of 16S rRNA methylases and extended-spectrum β-lactamases in AmpC-producing *Enterobacter cloacae*, *Citrobacter freundii* and *Serratia marcescens* in Korea

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Sir,

Enterobacter cloacae, *Citrobacter freundii* and *Serratia marcescens* are important nosocomial pathogens. In Korea, extended-spectrum β-lactamase (ESBL) prevalence in AmpC-producing *E. cloacae*, *C. freundii* and *S. marcescens* is quite high (10.9–23.6%), and these ESBL-producers show higher resistance rates to aminoglycosides than do the ESBL-non-producers.[1] While ribosomal protection mediated by methylation of 16S rRNA has been known as a self-defense mechanism for aminoglycoside-producing actinomycetes, it was not reported in other species until 2003. However, a series of methylases have been identified in several nosocomial pathogens, including *Pseudomonas aeruginosa*,[2] *S. marcescens*,[3] *Proteus mirabilis*[4] and *Klebsiella pneumoniae*.[5] The existence of these enzymes (RmtA, RmtB, RmtC and ArmA) is of great concern because they are capable of conferring an extraordinary high level of resistance (MIC > 512 mg/L) against most clinically important aminoglycosides, and they were often associated with ESBLs.[6]

In the present study, a total of 413 consecutive, non-duplicate isolates, including *E. cloacae* (158), *C. freundii* (126) and *S. marcescens* (129), were collected during March–July 2003 at 11 university hospitals in Korea. The isolates were from wound (37%), urine (35%), respiratory specimen (20%), blood (4%) and body fluid (4%). The MICs of amikacin (8–512 mg/L) and arbekacin (8–512 mg/L) were determined by an agar dilution method in accordance with the CLSI guideline. The detection of ESBL was based on the double disc synergy test (DDST) using discs containing 30 μg of ceftazidime, cefotaxime, aztreonam and cefepime. They were placed 2 cm from a disc containing amoxicillin/clavulanic acid (20/10 μg) (BBL, Cockeysville, MD, USA).

For the isolates that showed high-level resistance (MICs of >512 mg/L) to amikacin or arbekacin, a search for the 16S rRNA methylase genes (*rmtA*, *rmtB*, *rmtC* and *armA*) was performed by PCR. The total DNAs were extracted from isolates by boiling and the PCR was carried out with the *Taq* DNA polymerase (Takara Shuzo, Shiga, Japan) and the following sets of primers: rmtA-F, 5′-CTA GCG TCC ATC CTT TCC TC-3′; rmtA-R, 5′-TTT GCT

Table 1. Distribution of ESBLs among 16S rRNA methylase-producing *E. cloacae*, *C. freundii* and *S. marcescens*

	E. cloacae (13)	*C. freundii* (13)	*S. marcescens* (21)
ESBL producers	13	10	14
CTX-M-3	9	4	12
CTX-M-9	0	1	0
CTX-M-14	0	1 (*rmtB*)	1
TEM-52	1	0	0
SHV-12	2	1	1
PER-1	0	0	0
undetermined	1	3	0

TCC ATG CCC TTG CC-3′; rmtB-F, 5′-CCC AAA CAG ACC GTA GAG GC-3′; rmtB-R, 5′-CTC AAA CTC GGC GGG CAA GC-3′; rmtC-F, 5′-CGA AGA AGT AAC AGC CAA AG-3′; rmtC-R, 5′-ATC CCA ACA TCT CTC CCA CT-3′; armA-F, 5′-AGG TTG TTT CCA TTT CTG AG-3′; armA-R, 5′-TCT CTT CCA TTC CCT TCT CC-3′. Plasmids harbouring each gene were used as positive controls. For the detection of the ESBL genes, primers specific for bla_{TEM} (TEM-F, 5′-ATA AAA TTC TTG AAG AAA-3′; TEM-R, 5′-GAC AGT TAC CAA TGC TTA ATC-3′), bla_{SHV} (SHV-F, 5′-TGG TTA TGC GTT ATA TTC GCC-3′; SHV-R, 5′-GGT TAG CGT TGC CAG TGC T-3′), bla_{CTX-M} (CTX-M-F, 5′-CGC TTT GCG ATG TGC AG-3′; CTX-M-R, 5′-ACC GCG ATA TCG TTG GT-3′), $bla_{CTX-M-9}$ (CTX-M-9-F, 5′-CGC TTT ATG CGC AGA CGA-3′; CTX-M-9-R, 5′-GAT TCT CGC CGC TGA AGC-3′) and bla_{PER-1} (PER-1-F, 5′-AAT TTG GGC TTA GGG CAG AA-3′; PER-1-R, 5′-ATG AAT GTC ATT ATA AAA GC-3′) were used. PCR products were purified with a QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and sequenced on a 3730 DNA analyser (Applied Biosystems, Foster City, CA, USA). The nucleotide and deduced protein sequences were analysed with software available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Of the total 413 isolates, 58 were resistant to amikacin, and the majority (49/58, 84.5%) of these 58 isolates showed high-level resistance (MIC > 512 mg/L) to both amikacin and arbekacin. One *E. cloacae* isolate was highly resistant to arbekacin (MIC > 512 mg/L), but it was susceptible to amikacin (16 mg/L). The frequency of the high-level resistance to amikacin or arbekacin was 9.5% (15/158), 10.3% (13/126) and 17.1% (22/129) for *E. cloacae*, *C. freundii* and *S. marcescens* isolates, respectively, and almost all of them (13 *E. cloacae*, 12 *C. freundii* and 21 *S. marcescens* isolates) harboured the *armA* gene. One *C. freundii* isolate harboured the *rmtB* gene. The 16S rRNA methylase-harbouring isolates were isolated from nine hospitals distributed nationwide. All of them were highly resistant to both arbekacin and amikacin. None harboured an *rmtA* or *rmtC* gene. The ESBL production rate was significantly higher in 16S rRNA methylase-producers (100%, 76.9% and 66.7% among *E. cloacae*, *C. freundii* and *S. marcescens*, respectively), compared with 16S rRNA methylase-non-producers (25.0%, 12.4% and 10.2%, respectively) ($P = 0.002$, $P < 0.001$ and $P < 0.001$, respectively). Most of the ArmA producers co-harboured various ESBLs (CTX-M-3, CTX-M-9, CTX-M-14, TEM-52 and SHV-12), among which CTX-M-3 was the most common (Table 1).

This finding corroborates the previous reports that the *armA* was frequently associated with bla_{CTX-M} and they were co-transferred by conjugation.[6,7] Although only one isolate harboured the *rmtB* gene in the present study, it also harboured CTX-M-14; this coincides with the report by Yan *et al.*[6] where six out of the seven *rmtB*-positive isolates harboured CTX-M-14. In Korean medical practice, arbekacin is only rarely used for the treatment of methicillin-resistant *Staphylococcus aureus*. Nevertheless, the prevalence of high-level resistance to amikacin and arbekacin was similarly high, suggesting that *armA* can confer resistance to arbekacin as in other 16S rRNA methylases.[2,3]

In conclusion, the *armA* gene is widespread in Korean isolates of *E. cloacae*, *C. freundii* and *S. marcescens*, and an *rmtB* producer was also found. Given the multiresistance in these isolates, prudent antibiotic use, accurate detection of this resistance and strict infection control are urgently needed to prevent the spread of these organisms.

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Transparency declarations

None to declare.

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Dissemination of 16S rRNA methylase-mediated highly amikacin-resistant isolates of *Klebsiella pneumoniae* and *Acinetobacter baumannii* in Korea

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Abstract

Novel 16S rRNA methylase-mediated high-level resistance to amikacin and arbekacin has been reported recently in clinical isolates of Gram-negative bacilli only from several countries. We tested amikacin- or arbekacin-nonsusceptible Gram-negative bacilli isolated in 2003 and 2005 at a tertiary-care hospital in Korea by polymerase chain reaction to detect 16S rRNA methylase genes. *armA* alleles were detected in 14 isolates of *Klebsiella pneumoniae*, 10 other species of Enterobacteriaceae, and 16 *Acinetobacter baumannii*, whereas the *rmtB* allele was detected in 1 *K. pneumoniae* isolate. The resistance 1st detected in 2003 persisted in 2005. 16S rRNA methylase-producing isolates were highly resistant to arbekacin and amikacin, and were mostly coresistant to levofloxacin. Most *K. pneumoniae* isolates also produced extended-spectrum β-lactamases and plasmid-mediated AmpC β-lactamases, and most *A. baumannii* isolates were nonsusceptible to carbapenems.

Keywords: 16S rRNA methylase; *armA*; *rmtB*; High-level aminoglycoside resistance

1. Introduction

Aminoglycosides are used in the treatment of a broad range of life-threatening infections caused by both Gram-positive and aerobic Gram-negative bacteria (Mingeot-Leclercq et al., 1999). Activity of aminoglycosides depends on binding to a highly conserved motif of 16S rRNA (Yoshizawa et al., 1999). Mechanisms of aminoglycoside resistance include decreased outer membrane permeability (Hancock, 1981), active efflux (Hooper, 2005), amino acid substitutions in ribosomal proteins, and enzymatic modifications of the drug (Shaw et al., 1993). It could be expected that amikacin-resistant clinical isolates are rare, because amikacin was developed to block the access of a variety of aminoglycoside-modifying enzymes to their target sites (Kondo and Hotta, 2005). It was reported that among the strains collected in 1998 to 2003 in North America, 99.4% of Enterobacteriaceae and 85.2% of *Acinetobacter* isolates were susceptible to amikacin (Sader et al., 2005). The latest semisynthetic aminoglycoside, arbekacin, is active against methicillin-resistant *Staphylococcus aureus* (You et al., 2000) and has been extensively used in Japan since its approval in 1990 to treat methicillin-resistant *S. aureus* infections (Kondo and Hotta, 2005).

Galimand et al. (2003) reported that a *Klebsiella pneumoniae* isolate with a putative 16S rRNA methyltransferase was highly resistant to a large number of aminoglycosides. They named the 1st 16S rRNA methylase gene *armA*. The 16S rRNA methylase confers high-level resistance to 4,6-substituted deoxystreptamines, including arbekacin, amikacin, kanamycin, tobramycin, and gentamicin, by posttranscriptional methylation of rRNA leading to loss of affinity for aminoglycosides.

The *armA* gene is borne by a conjugative plasmid and has the potential to transfer horizontally (Galimand et al., 2003).

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Dissemination of the *armA* gene to various species of Enterobacteriaceae was reported in European countries (Galimand et al., 2005), and the gene was also detected in an *Escherichia coli* isolated from an animal (Gonzalez-Zorn et al., 2005). Other 16S rRNA methylase genes, *rmtA* and *rmtB*, were detected in *Pseudomonas aeruginosa* isolates (Yokoyama et al., 2003; Yamane et al., 2004), and in a *Serratia marcescens* isolate, respectively (Doi et al., 2004). The reported isolates with these genes were small in number, but in a Taiwanese study, *armA* and *rmtB* was detected in 12 and 2 isolates of *E. coli*, respectively, and 16 and 5 isolates of *K. pneumoniae*, respectively (Yan et al., 2004).

The *rmtA* gene-carrying *P. aeruginosa* isolate was highly resistant to amikacin and arbekacin (Yokoyama et al., 2003), suggesting usefulness of the resistance for screening the gene-carrying isolates. However, Gram-negative bacilli are usually not tested for arbekacin susceptibility in clinical laboratories, and the usefulness of amikacin resistance for screening *armA* gene-carrying isolates of Enterobacteriaceae and *Acinetobacter* spp. is unknown. In a Korean nationwide surveillance, the amikacin resistance rates of *K. pneumoniae* increased from 8% in 1997 to 13% in 2003 (Lee et al., 2006a). At a Korean tertiary-care hospital in 2004, resistance rates of *K. pneumoniae*, *S. marcescens*, *P. aeruginosa*, and *Acinetobacter* spp. to amikacin were very high, that is, 46%, 27%, 30%, and 65%, respectively (unpublished data), suggesting the possible presence of 16S rRNA methylase-producing strains.

It was reported that the *armA* gene is flanked by putative transposable elements and is linked to bla_{CTX-M}, suggesting the cotransfer of resistance to broad-spectrum cephalosporins (Galimand et al., 2003). In a Taiwanese study, most, but not all, of the *armA*- or *rmtB*-positive *E. coli* and *K. pneumoniae* isolates had CTX-M type enzymes (Yan et al., 2004), suggesting that this extended-spectrum β-lactamase (ESBL) gene is not always associated with *armA* or *rmtB* genes.

The aim of this study was to determine the prevalence of the 16S rRNA methylase gene in amikacin- or arbekacin-nonsusceptible clinical isolates of Enterobacteriaceae, *P. aeruginosa*, and *Acinetobacter* spp. and to characterize the isolates as to the aminoglycoside resistance level, the coresistance to β-lactams and fluoroquinolone, and the molecular epidemiology. The usefulness of amikacin and arbekacin disk diffusion test in screening for the isolates containing *armA* gene was also determined.

2. Materials and methods

2.1. Bacterial strains and antimicrobial susceptibility testing

Gram-negative bacilli were isolated from clinical specimens at a Korean tertiary-care hospital. The species were identified by conventional methods (Farmer, 2003; Schreckenberger et al., 2003) or by using the Vitek GNI or ATB 32 GN systems (bioMerieux, Marcy L'Etoile, France). In the 1st phase of this study in September 2003, consecutive nonduplicate isolates by each indicated species were used, and in the 2nd phase study in June 2005, 216 consecutive nonduplicate isolates of all indicated species were used.

The antimicrobial susceptibility was tested by the disk diffusion test (CLSI, 2006) using commercial disks and Mueller–Hinton II agar (Becton Dickinson, Sparks, MD). Arbekacin susceptibility was tested by using 30-μg disks (Eiken Chemical, Tokyo, Japan) and interpreted as recommended by the disk manufacturer: ≤13 mm, resistant; and ≥18 mm, susceptible. MICs of aminoglycosides were determined by the agar dilution method (CLSI, 2006) using arbekacin (Meiji Seika, Tokyo, Japan), amikacin and kanamycin (Dong-A Pharmaceutical, Seoul, Korea), gentamicin and tobramycin (Dong Wha Pharmaceutical, Seoul, Korea), isepamicin and netilmicin (Yuhan, Seoul, Korea), streptomycin (Chong Kun Dang Pharmaceutical, Seoul, Korea), and spectinomycin (Kuk Je Pharmaceutical, Seoul, Korea). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used for quality control.

2.2. Detection of the 16S rRNA methylase gene by polymerase chain reaction

To detect *armA*, *rmtA*, and *rmtB* gene alleles, we tested isolates with arbekacin MIC ≥16 μg/mL in the 1st phase, whereas in the 2nd phase, isolates nonsusceptible to arbekacin or amikacin disk diffusion test were used. Polymerase chain reaction (PCR) primers used for *armA* and *rmtA* were as those previously reported (Yokoyama et al., 2003; Yamane et al., 2005), and others are shown in Table 1. The reaction was carried out in a total volume of 20 μL, containing 1 μL of template and PreMix (Bioneer, Cheongwon, Korea). Amplification conditions were the following: for 16S rRNA methylase genes, 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 53 °C (*armA*), 58 °C (*rmtA*), or 60 °C (*rmtB*) for 30 s, and 72 °C for 45 s, and a

Table 1
Primers used for PCR detection of 16S rRNA methylase genes and β-lactamase genes

Primer	Target	Oligonucleotides (5′ to 3′)	Expected size (bp)	Reference
RMTB-F	*rmtB*	CCC AAA CAG ACC GTA GAG GC	584	This study
RMTB-R		CTC AAA CTC GGC GGG CAA GC		
CTX-MUNI-F	bla_{CTX-M}	CVA TGT GCA GYA CCA GTA A	585	A. Baumfeind[a]
CTX-MUNI-R		ARG TSA CCA GAA YMA GCG G		
CTX-M14-F	$bla_{CTX-M-14}$	ACA ATG ACG CTG GCA GAA CTG	512	This study
CTX-M14-R		TTA CAG CCC TTC GGC GAT GA		

[a] Personal communication.